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# The Effect of Climatic Conditions on the Growth of Barley.

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With six Figures in the Text.

THE relation of climate to the growth of the plant has important bearings on many problems of scientific as well as economic interest. It is, for instance, impossible to interpret the effect of manures on crop yield by an experimental method consisting of repetitions year by year of field trials, unless variations in climate are taken into account in assessing the results of such experiments.

The production also of new varieties suitable for local climatic conditions must always remain a matter of pure empiricism, until the interaction of the climatic complex with the physiological processes of distinct races is understood. Furthermore, the application of physiology to agricultural problems seems to the author to find its most fertile field in disentangling the effect of external factors from the general interplay of processes determining the yield of the plant. It may confidently be expected that in this way the necessary experimental evidence required to check knowledge acquired by laboratory methods, where external factors are as far as possible varied one at a time, will be obtained.

Investigations of this kind are singularly lacking, in spite of the fact that this aspect of physiological problems led to a considerable deal of work in Germany, mainly between 1877 and 1880, under the inspiration of Kreuzler. A great mass of data was at this time accumulated, but methods of dealing with the experimental results had not then been developed, and until recent years the information thus collected was valueless to science.

The subject has been reopened recently, and a paper dealing with the problem on these lines was published in 1920, embodying the analysis by R. A. Fisher, in collaboration with W. F. Brenchley, of data on the growth of *Pisum sativum*.

The author has been interested since 1917 in problems of this kind, and in that year published in the Report of the Cheshunt Exp. Station an outline of a method of analysis of plant growth, together with figures of net assimilation rates calculated from dry weight and leaf-area data. The method there developed has since been employed and elaborated by Kidd, West, and Briggs.

#### EXPERIMENTAL METHOD.

A pure line Goldthorpe barley has been grown in pot culture at Rothamsted each year since 1921. The soil used was obtained from one of the experimental fields (Little Hoos) and before use was sieved and mixed with 10 per cent. of sand. The pots were of glazed earthenware, measuring 10 in. by 10 in., holding approximately 30 lb. of soil. They were provided below with a tubulure which was closed with a cork carrying a glass tube with a right-angled bend leading into a glass vessel to catch drainage water. At the time of filling the pots, the water content of the soil was determined and exactly 30 lb. of soil were weighed into each pot. Sufficient water was then added to bring the water content up to 15 per cent., which was known to be optimum for this particular soil. Throughout the experiments the aim was to maintain the water content at this figure, and although higher water contents could not be prevented, since the pots were exposed on the roof of the laboratory, 15 per cent. represented the lower limit. To ensure this end, each pot was weighed twice a week during dry weather and the water lost by evaporation was replaced, either from a tank of tap-water kept at air temperature or from the bottles of 'percolated' water.

The seeds before planting were first selected by eye for uniformity and then graded by weight between the limits 50–60 mg. Six seeds were sown in each pot, of which three were subsequently removed, leaving the three most uniform seedlings. For reasons to be explained subsequently, two types of manurial treatment were used, *A* consisting of 0.5 gm.  $\text{NaNO}_3$ , 1 gm. superphosphate, 0.25 gm.  $\text{K}_2\text{SO}_4$  per plant, and the other, *B*, differing in having 1 gm.  $\text{NaNO}_3$  and 1 gm.  $\text{KNO}_3$  per plant. In spite of the manurial differences all the series of experiments have been utilized for the purpose of this paper. This was possible since, as will be seen later, the assimilation rates in the two series were identical, and correction was made for the final differences in dry weight and leaf-area.

The manurial treatments and phenological data are given in the following table :

TABLE I.

Year.	Manurial Treatment.	Sowing Date.	Germination Date.	Harvest.
1921	A.	May 3	May 10	Aug. 9
1922	A.	May 8	May 17	Oct. 2
"	B.	May 16	May 22	Oct. 2
1923	A.	May 1	May 7	Sept. 12
1924	B.	April 16	April 23	Aug. 25

Six pots were removed each week, the soil washed out from the roots by a stream of water, the leaf-area measured with a planimeter, and the plants subsequently dried in an electric oven at 102° C. Before drying the plants were separated into roots, stems (including leaf sheaths), dead leaves, green leaves, and ears, each of which were weighed separately. The dry material thus obtained was afterwards ground and used for determination of total nitrogen, ash, and calorific value. These data will be considered in a subsequent paper. The considerations here put forward will deal only with leaf area and data of total dry weight.

The primary plant data thus collected provided information on the dry weight and leaf-area of the average of eighteen plants, week by week throughout the growing season. It is not necessary here to discuss fully the accuracy of these data, but it may be said that the probable error of the average dry weight rarely exceeded  $\pm 2$  per cent., and for many samples was below  $\pm 1$  per cent. The data for leaf-area were somewhat less reliable, as in later samples it was found impossible to measure more than three individual plants, and the mean leaf-area had to be calculated from the relation established between leaf-area and leaf weight. From these primary data the net assimilation rate was calculated in a manner to be explained later.

#### ANALYSIS OF THE DATA.

For purposes of analysis three measures of growth have been used:

- (1) Net assimilation rate.
- (2) Relative growth rate of the leaf surface.
- (3) Relative rate of increase in dry weight. (Efficiency index.)

The meteorological data with which these have been correlated were collected *in situ*, and consisted of average day temperature, average night temperature, total solar radiation, hours of bright sunshine, and the evaporating power of the air. The temperatures were derived from continuous thermograph records by averaging two-hourly readings on the charts for day and night throughout the season. Radiation was measured with a Callendar self-recording radiometer, the record for each day being

integrated with a planimeter. The evaporation data were obtained from a porcelain atmometer belonging to the Rothamsted Experimental Station, fully exposed to rain on the laboratory roof; hence these last data can scarcely be considered satisfactory.

### CLIMATIC CONDITIONS.

The variation of climate encountered during the course of the experiment is considerable. 1921 will long be remembered as a year of continuous sunshine and lack of rain. 1922 was characterized by a spell of fine weather immediately after germination, giving way later to almost continuous dull and rainy weather. In 1923 and 1924 conditions were almost the antithesis of these, the weather at first being dull and cold and improving later. Full details of the climatic conditions are recorded in the following table:

TABLE II.

Month.	Max. Temperature.				Min. Temperature.				Hrs. Bright Sunshine.				Rainfall (inches .)			
	1921	1922	1923	1924	1921	1922	1923	1924	1921	1922	1923	1924	1921	1922	1923	1924
May	62.1	65.4	56.7	61.1	43.3	44.7	42.0	45.4	209	280	166	191	1.445	1.579	1.681	4.628
June	67.5	65.9	60.7	65.2	47.6	48.1	46.8	50.2	216	229	116	200	0.194	1.038	0.617	1.974
July	77.0	63.7	72.5	68.3	53.4	49.7	55.1	51.0	240	150	224	236	0.179	4.605	3.871	4.533
August	69.2	63.2	68.5	64.8	52.6	49.2	51.0	50.5	145	127	257	169	1.113	2.930	2.329	2.351
September	—	60.5	62.9	—	—	46.3	46.2	—	—	10.3	180	—	—	2.882	2.541	—

In dealing with the complex relations between the measures of growth and the changing environmental conditions, the ordinary method of drawing graphs indicating the relation between the variables fails, since it is obvious that over periods as long as a week all the factors have been conditioning the rates of the processes, and the effects of the individual factors obscure each other. The relations sought for can, however, be determined by the method of correlation. The assumption is made that, had the magnitude of all environmental factors remained at the mean value, the rate of the process studied (net assimilation rate, relative leaf growth rate, &c.) would have remained at its mean value for the whole period. Any change in the rate of the process is assumed to be due to the change from their mean values of one or more of the external factors. The method deals with these departures from the mean values, and investigates the extent to which they are associated.

Difficulties immediately arise:

1. The external factors themselves do not vary independently, as, for example, solar radiation and temperature-level tend to vary together. By taking 'partial correlations' this difficulty is overcome, and allowance is made for the relations inherent among the environmental conditions, in assessing the effect of each, singly, on the rate of the process studied.

2. The assumption is made that, in so far as variations in the level of the external factor affect the rate of the process at all, small or large variations will have proportionate effects. This is by no means true, as an optimum value for external factors acting in combination undoubtedly exists. In spite of this, however, the trend of the interrelations within the range of conditions explored will appear from the analysis, unless any of the factors have an optimum at the mean value, in which case the partial correlation coefficient may be zero and the rise and fall in the rate of the process will be obscured. Also, clearly, it will be inadmissible to extrapolate outside the range of values actually explored.

3. Even were the external factors to remain constant at their mean values, the rate of such a process as relative dry weight increase would not remain constant at its mean value. In fact, the rate of this process is not solely conditioned by external factors, but depends also on the action of varying internal factors which independently determine the rates at successive moments. The method devised to overcome this difficulty will be described later. The net assimilation rate does, however, appear to fulfil the stipulated condition.

It is necessary to stress first of all the importance of the numerical measures selected for evaluating the external factors. It is desirable to use quantities which are easily measured, and for this reason maximum and minimum temperatures and hours of bright sunshine are ideal measures of climate, and indeed are the characteristics generally selected for meteorological records. For physiological purposes they are singularly unsatisfactory. An indispensable characteristic of climatological indices is that the distribution in time of the intensity of the factor measured shall be included in the standard. Integrated thermograph records, or, what is almost equivalent, averages of two-hourly readings of the chart, fulfil this requirement, as do also integrated records of the Callendar radiometer, and the values for radiation derived from the latter, share with the former the advantage that all intensities are recorded, and not maximal intensities only, as in records of bright sunshine.

It is not the author's intention to discuss the whole question of climatological indices in this connexion, but it may be pointed out that Livingstone's<sup>1</sup> suggestion of using averages of temperature, weighted according to growth rates of specified plants observed under laboratory conditions, cannot simplify the problem of determining the relation of plant growth to the climatic complex and the part played therein by the temperature factor. It is precisely the change in the relation of growth to the single factor, brought about by the interaction of other factors, that needs to be known, and

<sup>1</sup> B. E. Livingstone : *Temperature Coefficients in Plant Geography and Climatology*. Bot. Gaz., lvi, pp. 349-75, 1913.



can only be derived from the primary data. It must be realized that it is not necessary in the calculation of correlations to restrict oneself to the first power of the variables, such as temperature, and that by utilizing higher powers, such as  $t^2$  or  $t^3$ , &c., and treating these as independent variables for calculating partial correlations, the relation between the rate of the process studied and external factors may be expressed with any degree of nicety, and the regression lines may be curves of any form. In this way optima may be located with a degree of precision depending only on the patience of the computer and the quantity of data available. A paucity of data alone made such treatment in this case unprofitable.

### *The Interrelation of Climatic Factors.*

The mean values for the climatic conditions during the periods of the experiments are summarized below :

	<i>Mean.</i>	<i>Standard Deviation.</i>
A. Mean max. temp.	64.94° F.	4.567° F.
B. „ min. „	47.94° F.	3.259° F.
C. Mean hours sunshine per week	44.76 hours	18.026 hours
1. Average day temp.	57.08° F.	3.858° F.
2. „ night „	52.07° F.	3.033° F.
3. Total radiation in cals. per sq. cm. per week	2513 cals.	507.4 cals.
6. Evaporating power of the air (grm. evaporated per hour)	0.4321 grm.	0.2915 grm.

The extent to which these factors are interrelated is shown by the following correlation coefficients, calculated from thirty-one pairs of values :

$$\begin{array}{ll}
 {}^1r_{AB} = +0.590 \pm 0.119 & r_{12} = +0.916 \pm 0.029 \\
 r_{AC} = +0.676 \pm 0.099 & r_{13} = +0.640 \pm 0.108 \\
 r_{BC} = +0.057 \pm 0.182 & r_{23} = +0.425 \pm 0.150
 \end{array}$$

Maximum day temperature and minimum night temperature are much less closely correlated than are average day and average night temperature, though in both cases the factors vary together. The reason for the large difference in correlations between radiation and minimum temperature becomes clear from the partial correlation as shown below.

$$\begin{array}{ll}
 r_{AB.C} = +0.750 & r_{12.3} = +0.926 \\
 r_{AC.B} = +0.797 & r_{13.2} = +0.692 \\
 r_{BC.A} = -0.575 & r_{23.1} = -0.525
 \end{array}$$

${}^1r_{AB}$  signifies the correlation between the two variables *A* and *B*. When partial correlations are used the first two symbols preceding the point represent the two variables under consideration, while the symbols following the point indicate the factors whose effect has been eliminated. Thus  $r_{AB.CD}$  indicates that four factors have been studied, and represents the correlation between the two factors *A* and *B*, when the effects of *C* and *D* have been eliminated.

When the effect of the third factor has been allowed for, the correlation between day temperature and night temperature rises, while the true relation between night temperature and radiation is found to be inverse; in other words, after allowance has been made for the tendency of warm nights to follow warm days, and sunny days to be associated with high temperatures, it is seen that sunny days tend to precede cold nights. This is undoubtedly due to the absence of cloudiness leading to rapid heat loss at night by radiation. Correlations similar in sign were found to hold in the greenhouse by Dr. W. E. Brenchley.<sup>1</sup> The closer association of maximum temperature with hours of bright sunshine than average temperature with total radiation is to be expected, since a high maximum temperature may be reached even during a short period of bright sunshine, which will not greatly affect the average temperature over the day.

The partial correlations with evaporating power of the air are given below :

$r_{16\cdot23} = +0\cdot429$	1. Average day temperature.
$r_{26\cdot13} = -0\cdot373$	2. Average night temperature.
$r_{36\cdot12} = +0\cdot212$	3. Total radiation.
	6. Evaporating power of the air.

As would be expected, high temperature is associated with high evaporation, but the relatively large effect of high radiation, even after correction for associated temperature, is less easy to interpret, as is also the inverse relation with night temperature. Undoubtedly both exert their action through associated rainfall. The evaporimeter used was not screened from the weather, and hence rain reduced the amount of evaporation registered, apart from the higher humidity prevailing. The positive partial correlation with solar radiation thus indicates the absence of rain at such times, and the negative partial correlation with night temperature signifies only that cloudless nights tend to be colder and are free from rainfall.

### *The Effect of Climatic Conditions on Net Assimilation Rate.*

'Net assimilation rate' may be defined as the increase in weight of the dry material of a plant per unit leaf-area per unit time (unit leaf rate, Briggs, Kidd, and West<sup>2</sup>), and is calculated from the difference in dry weight of average plants at the beginning and end of a given period of time, divided by the average area of the leaf surface during the period. Where observations of leaf-area are made at frequent intervals between the times of sampling, the mean area can be ascertained readily by integrating the curve

<sup>1</sup> W. E. Brenchley : On the Relations between Growth and the Environmental Conditions of Temperature and Bright Sunshine. *Ann. App. Biology*, vol. vi, pp. 211-44, 1920.

<sup>2</sup> C. West, C. E. Briggs, and F. Kidd : Methods and Significant Relations in the Quantitative Analysis of Plant Growth. *New Phytologist*, xix, pp. 200 et seq., 1920.

of leaf area drawn through the experimental points. The net assimilation rates obtained in this way for cucumbers were published by the author in 1917.<sup>1</sup> When information is confined to areas obtained from samples at the beginning and end of a given period of time, the problem of ascertaining the mean area during the period is more difficult. The mean area will clearly be the increment in area divided by the mean relative leaf growth rate.

The problem of determining mean relative growth rates in such cases has been fully dealt with by R. A. Fisher,<sup>2</sup> who has indicated the general method of calculation. The mean areas were obtained by dividing the difference in area at two successive times of sampling by the difference of their napierian logarithms. From each successive pair of samples the net assimilation rates were calculated by dividing the increment in dry weight by the average leaf-area obtained by the above method.

The first part of the whole growth period alone was used for calculating assimilation rates, namely, until the maximum leaf-area had been attained. The reasons for confining attention to this period only are that, firstly, after this time the leaves begin to die off rapidly, and to discriminate between functionally green leaves and dying leaves becomes progressively more difficult; secondly, the stems after this point begin rapidly to elongate and an increasingly large part of total assimilation may be due to their activity.

The factors involved in the analysis are :

1. Average day temperature.
2. Average night temperature.
3. Total radiation.
4. Net assimilation rate in grm. per sq. dm. per week.

$$r_{14 \cdot 23} = +0.394, \text{ significance } 37 : 1.$$

$$r_{24 \cdot 13} = -0.434, \quad \text{,,} \quad 50 : 1.$$

$$r_{34 \cdot 12} = +0.429, \quad \text{,,} \quad 50 : 1.$$

The partial correlations of average day temperature with assimilation rate and total radiation with assimilation, after correcting for the effects of the other two factors, are positive, but the effect of radiation predominates over that of temperature. The negative sign and the magnitude of the correlation with average night temperature are striking. From the fact that net assimilation rate takes no account of respiration, and that the latter increases with average night temperature, while the former is in abeyance, an inverse relation with average night temperature would be expected, but the magnitude of the effect is greater than would be inferred from this, since the length of the night period during the summer is so much shorter than the day.

<sup>1</sup> F. G. Gregory : Third Annual Report, Experimental and Research Station, Cheshunt, 1917.

<sup>2</sup> R. A. Fisher : Some Remarks on the Methods formulated in a Recent Article on 'The Quantitative Analysis of Plant Growth'. Ann. App. Biol., vii, pp. 367-72, 1921.

Partial correlations have also been calculated with maximum day temperature (*A*), minimum night temperature (*B*), and hours of bright sunshine (*C*), and are as follows :

$$r_{A,BC} = +0.544, \text{ significance } > 100:1.$$

$$r_{B,AC} = -0.500, \text{ significance } > 100:1.$$

$$r_{C,AB} = +0.182, \text{ not significant.}$$

The signs of the correlations are similar to those already given, but a striking change in value of the partial correlation with radiation appears. Radiation as measured in terms of hours of bright sunshine is much less closely associated with assimilation than when all intensities are taken into account. This may indicate one of two possibilities : either (1) assimilation rate has an optimum point below the highest intensities of radiation, measured as bright sunshine, or (2) the assimilation at low intensities of light and in diffuse light appears in these correlations as due to temperature effects with which they are correlated, and which have not been allowed for since such light intensities are not included in the measurements in the absence of bright sunshine. Assimilation will certainly proceed in the absence of bright sunshine, and all this will be credited to the concomitant temperature. The important bearing of the choice of standards of measurement of climatic factors appears very clearly in this connexion. The strong adverse effect of high night temperatures again appears in this set of correlations. The regression equation for the first set of partial correlations is stated below :

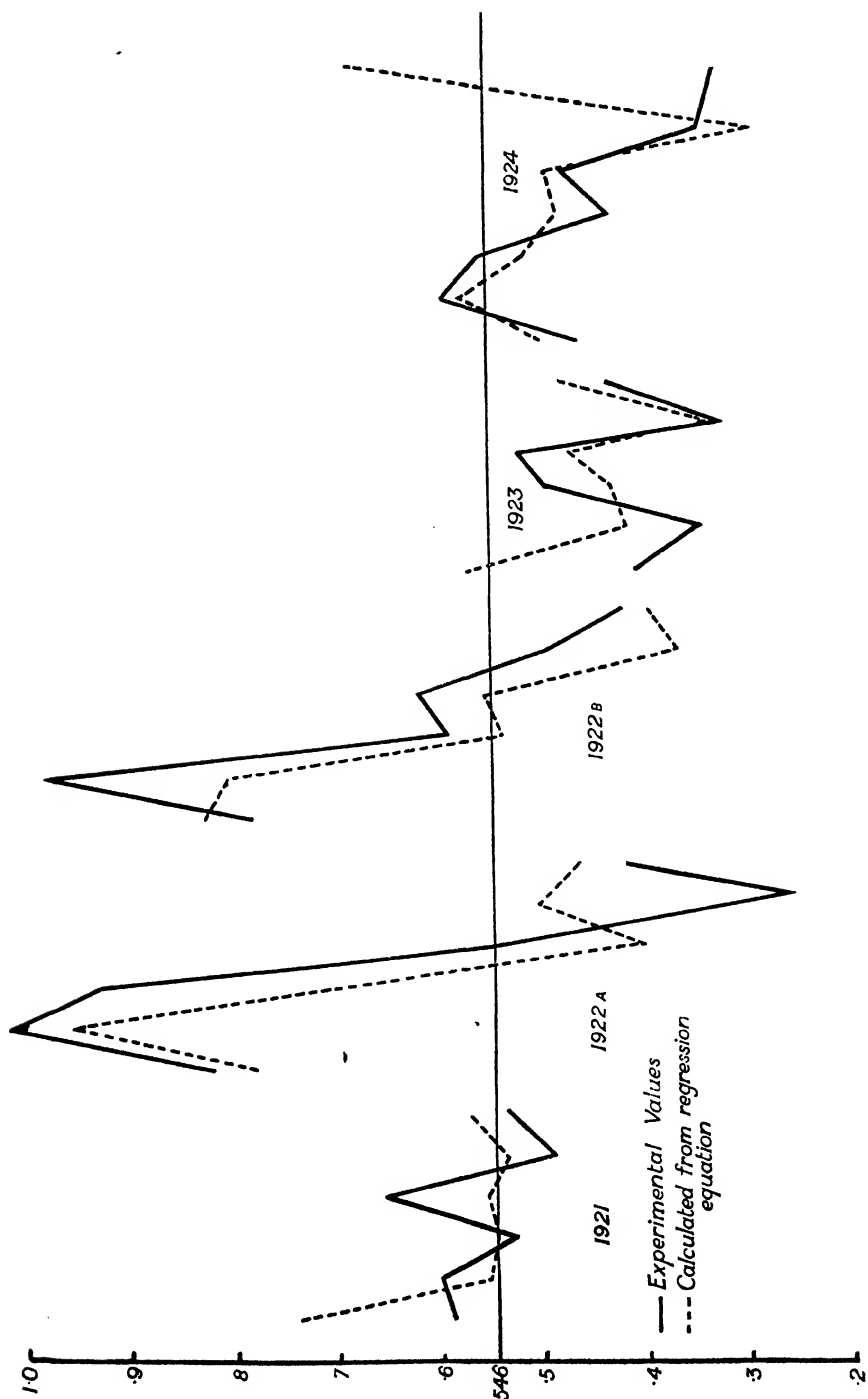
$$R_a = 0.0499 T - 0.0598 t + 0.1823 L + 0.3551,$$

where *T* is the average day temperature in degrees F., *t* the average night temperature, and *L* is total radiation in 1,000 calories per week. By substituting values of the three variables the net assimilation rate (*R<sub>a</sub>*) may be calculated for all combinations.

Fig. 1 represents the experimental and calculated values of assimilation rate for all the experiments. The agreement all over the range of conditions encountered is very striking. The horizontal line represents the mean assimilation rate of 0.546 grm. per sq. dm. per week, and it will be seen that the experimental and calculated values oscillate together about the mean value. The only large discrepancy is the final value for 1924, and corresponds with a sudden change in climatic conditions. After a period of dull weather with much rain, the weather suddenly became bright and hot, as is indicated in the sudden rise in the calculated value for assimilation. The plants had developed very large leaf surfaces, and the increased evaporation under more favourable conditions may have led to a condition of considerable water-strain. In addition to this, the soil had been saturated with

water for a considerable time, and the lack of aeration may have played a part. In the subsequent week the assimilation rate rose to its calculated value, and it is interesting to note that the dry weight per unit area of leaf rose from 0.338 to 0.352, indicating a rapid thickening of cell-wall. Over 80 per cent. of the variation in assimilation rate is accounted for by change in climatic conditions. Since the effects of other factors have been neglected, and also the effects of sampling error, the agreement with expectation is excellent. As leaf-area and dry weight are highly correlated in individual plants, the sampling errors introduced will tend to be small. It is remarkable that the effect of the omission of two important factors, namely, time and the quantity of nitrogen added as manure, in the analysis should be so small. As regards the first, there is no evidence that, up to the point in the life-cycle at which maximum leaf-area occurs, the net assimilation rate falls off with time, and, should there be such an age effect, it must be very small compared with the changes in assimilation with external conditions. Internal factors apparently play a negligible part in the regulation of the net assimilation rate.

With regard to the effect of nitrogenous manuring, the result is of great interest. Comparing the curves A and B for 1922 (Fig. 1), it is seen that the actual rates found experimentally agree equally well in the two cases with the calculated values, made on the assumption that nitrogen manuring has no effect on assimilation rate, although the quantities of nitrogen added differed by nearly 400 per cent. The maximum leaf-areas obtained, the final dry weights, and tiller numbers in the two cases, on the other hand, differed widely. From the first also a constant percentage difference of nitrogen was observed in the tissues of the plants in the two sets. Hitherto the mechanism by which nitrogen brings about an increase of final yield has not been clear. The larger leaf surface and deeper green colour with increasing nitrogen had been observed, but whether a change in assimilation rate takes place, or whether the larger yields were only due to a larger assimilating surface, was unknown. These results indicate strongly that, over the range of nitrogen manuring here employed, the whole effect on yield is due to the stimulating action of nitrogen on leaf growth, enabling greater total assimilation to take place without change in the net assimilation rate. If real assimilation is increased, then respiration must be correspondingly increased, or the net assimilation would not remain unaffected. The possibility of a very low level of nitrogen manuring depressing assimilation rate has been borne in mind, and experiments to test this point are in progress. In concluding this part of the subject, it may be said that net assimilation rate is almost completely controlled by the factors of temperature and radiation, and internal factors play a minor part. It must, however, be remembered that in these experiments the water relations have been more or less controlled, and in nature their part may sometimes be very significant. Control of



**FIG 1.** Comparison of experimental values of net assimilation rate with those calculated from regression equation.

net assimilation by temperature and light is almost equally close, and in general neither one nor the other can alone be limiting the process over any considerable period of time.

*The Effect of Climatic Conditions on Relative Leaf Growth Rate.*

The definition of relative leaf growth rate has already been given, and the method of calculation has been indicated. The shape of the curve of

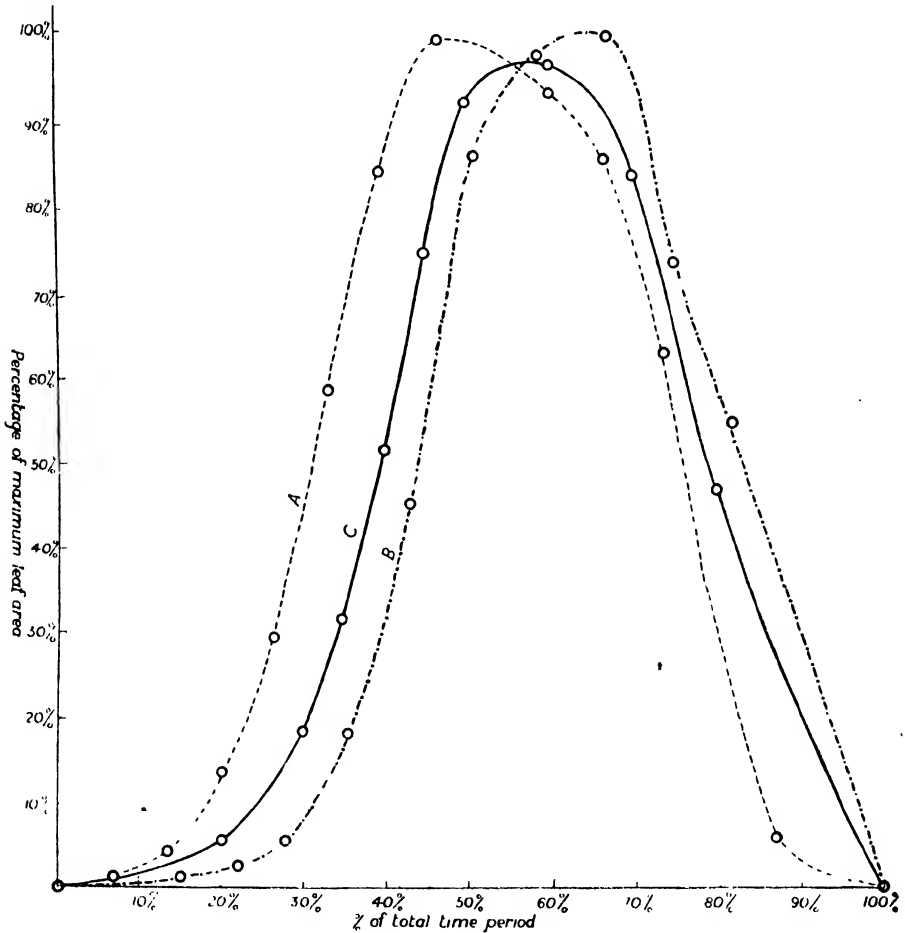


FIG. 2. A. Curve of total leaf-area for Experiment B of 1922. B. Curve of total leaf-area for Experiment of 1924. C. Mean curve (normal curve).

total leaf-area from the beginning up to the death of the leaves is shown in curves A and B, Fig. 2. The curve is of a complex shape, the elucidation of which is reserved for treatment in a subsequent paper. It is, however, obvious that the leaf-area is not a simple function of time, and the relative leaf growth rate varies in time in a complex manner. This precludes the

possibility of calculating straightforward correlations with time as one of the variables, since this would assume a linear time relation. By taking  $t^2$  and  $t^3$  as independent variables, this difficulty could be surmounted, although with great increase in labour. Other difficulties to be mentioned below would, however, still defeat the end.

As the same difficulty is met in dealing with relative dry weight

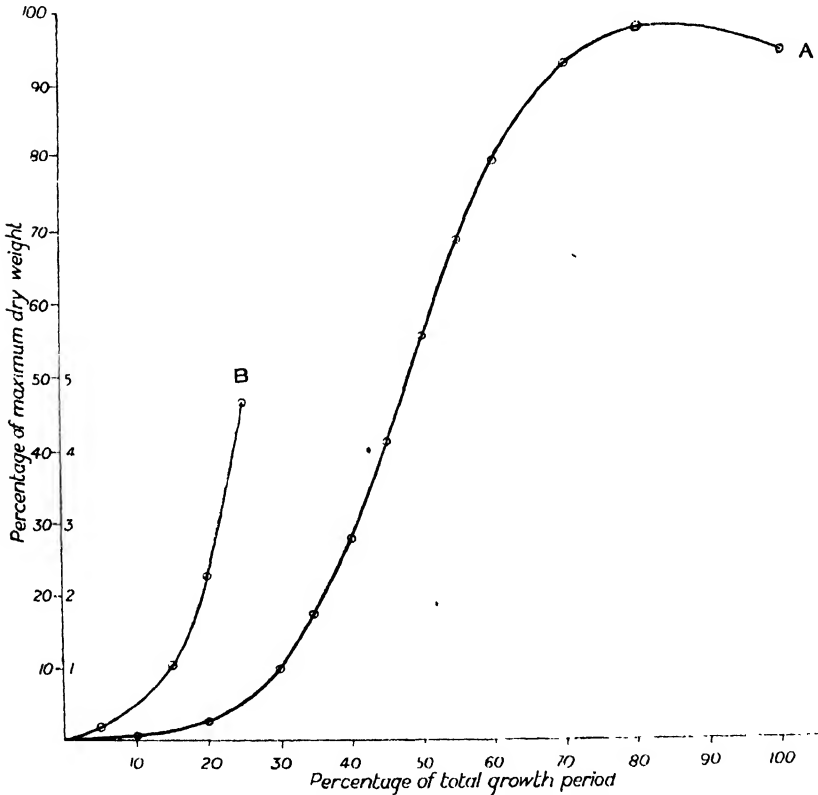


FIG. 3. A. Mean curve of total dry weight. B. First portion of curve A on larger scale.

increase, and the method of handling the problem in the two cases is identical, they will be discussed together here. The curve of dry weight increase is shown in Fig. 3.

If the environmental conditions remained constant at their average magnitudes, the shapes of the curves of leaf-area and dry weight would still remain of the same form. In other words, the succession of growth rates indicated by the changing slopes of the curves is determined largely by internal physiological factors. The effect of the environment is reflected in local changes in the slopes and by a shift in the points of inflexion and a change in the end points.



Under environmental conditions artificially maintained at a constant level the changing slope of the curve would be conditioned entirely by the changing internal factors of the plant. Such a curve might be called a 'normal curve'. The shape of the 'normal curve' will of course vary with the level at which the external factors are maintained; indeed this has been experimentally verified by the author for cucumbers growing under constant conditions of temperature and light.

If the shape of the 'normal curves', showing the changing rates of the two processes with time under constant average conditions, were known the effect of changes in the environment might be measured by comparing the slopes of comparable portions of the experimental and normal curves. This is essentially the method which has been utilized. The experimental curves for single experiments during the four years, however, differ among themselves in many respects. The period of the growth cycle varies from year to year, and the maximum points of the leaf-area and dry weight curves vary with the season and with the type of manuring used. All the curves have therefore been standardized by taking the period of the whole cycle as 100, and the maximum attained as 100, and re-calculating for each sample taken the time after germination as a percentage of the whole time, and the magnitudes of leaf-area and dry weight as percentages of the maximum value attained in the series to which they belonged. In this way the curves can all be presented on the same scale. Fig. 2 shows the two extreme curves for leaf-area derived from the experimental data of 1922 B and 1924 and treated in this way, as well as the normal (mean) curve obtained by averaging the values of single experiments.

In order to obtain an approximation to the 'normal curves', smooth curves were drawn through the experimental points representing each series, and the values of leaf-area and dry weight read off at comparable time intervals, viz. 10 per cent., 20 per cent., &c., of the whole period. Geometric means of the values so obtained were taken, and from these the mean curves (approximate 'normal curves') shown in Figs. 2 and 3 were constructed. Geometric means rather than arithmetic means were taken, as it was rates of growth which were to be averaged. The curves so obtained have not been smoothed, and show no marked irregularities.

These curves were treated as approximations to the normal curves required, and were taken to represent the succession of growth rates as determined by internal factors alone under a uniform environment. All that remained to be done was to re-calculate the value of each sample in all the series, taking the maximum value experimentally found and reading off the normal curve the percentage of this value corresponding with the percentage of the total time cycle which had elapsed when the particular sample in consideration was taken. In this way the experimental curves were restored to their original scale of time and magnitude and the correspond-

ing values of a similar normal curve were known. The napierian logarithms of each value for experimental and calculated normal series were taken, successive values for weekly samples in each case were subtracted, and thus the relative growth rates over comparable periods for experimental and normal curves were known. The differences between these relative rates measured the effects of changing environment. Thus, if the difference was zero, there was no effect, while acceleration and inhibition gave positive and negative values of the difference respectively. The process of calculation is shown for the leaf-area figures of 1921 in Table III. Only the portion of the curve previous to maximum leaf area was used, as already indicated. The whole time period, which was taken as 100, for relative leaf growth rate calculations was the number of days which elapsed from germination to the death of the leaves, but for efficiency index the whole life-cycle up to harvest was utilized.

TABLE III.

Days.	% Whole Period.	Actual Area.	Area calculated from Normal Curve.	Rel. Growth Rate.		Difference.
				Actual.	Calc. Normal.	
7	7.7 %	8.93 sq. cm.	89.9 sq. cm.			
14	15.4 %	28.9 "	32.4 "	1.1745	1.2820	-0.1075
21	23.1 %	90.3 "	91.2 "	1.1393	1.0350	+0.1043
28	30.8 %	190 "	229 "	0.7439	0.9206	-0.1767
35	38.5 %	479 "	521 "	0.9246	0.8221	+0.1025
42	46.2 %	787 "	832 "	0.4966	0.4680	-0.0289
49	53.8 %	899 "	881 "	0.0553	0.0573	-0.0018

The differences in the relative growth rates, such as are shown in the last column of Table III, were those utilized for calculating the correlation coefficients. The factors studied were :

1. Av. day temperature ( $T$ ).
2. Av. night temperature ( $t$ ).
3. Total radiation ( $L$ ).
4. Net assimilation rate ( $R_a$ ).
5. Relative leaf growth rate ( $R_e$ ).
6. Evaporating power of the air ( $E$ ).

The factors 1, 2, 3, and 5 were used for the first set of partial correlations, and the partial correlations are shown below, together with the regression equation :

$$r_{15.23} = +0.578, \text{ significance } > 100 : 1.$$

$$r_{25.13} = -0.474, \quad \text{,,} \quad 100 : 1.$$

$$r_{35.12} = -0.383, \quad \text{,,} \quad 21 : 1.$$

$$R_e = 0.1430 T - 0.1172 t - 0.2814 L - 1.3504. \dots (2).$$

As with net assimilation, the positive effect of day temperature and negative effect of night temperature appear, but a totally different effect of

radiation is seen. High radiation is *negatively* correlation<sup>2</sup> with relative leaf growth, which indicates that, after allowance has been made for the high temperature associated with light sunshine, the effect of strong radiation on leaf growth is inhibitory. Whether this holds over the whole range of light intensities cannot be ascertained from these data, but it is quite clear that for leaf growth the average intensity of light during the summer is in excess of the needs of the plant. Confirmatory evidence of this for barley is seen in certain experiments of Dr. W. E. Brenchley,<sup>1</sup> in which plants crowded together were compared with plants widely spaced. As each plant was grown singly in water culture there was no question of limited water or nutrients. The striking differences in leaf-area in the two sets are clearly seen in the published photographs. The conclusion that the effect was due to humidity differences is not conclusive; it may have been due to the reduction of light intensity in consequence of crowding leading to higher relative leaf growth rate in accord with the negative correlation here established. In view of the fact that in spite of a larger leaf surface the dry weights of the crowded plants fell much below that of the spaced, it is clear that the net assimilation rate fell with the low light intensity, as would be expected from the high positive correlation of net assimilation and light recorded above. An optimum for leaf growth is probable at low intensity of light; an investigation to test this possibility the author hopes shortly to undertake.

The magnitude of the negative correlation with night temperature is difficult to understand. It has been seen that radiation has an inhibitory effect on relative leaf growth, and hence it would be supposed that this process would be most active during the night. Whence, then, the negative correlation with night temperature? A possible explanation which presented itself was that the detrimental effect of high night temperature was bound up with the negative correlation of net assimilation rate with night temperature, and might be due to respiration losses. To test this hypothesis a new set of partial correlations are calculated, bringing in net assimilation rate as one of the variables.

The result is presented below :

1. Av. day temp.
2. Av. night temp.  $r_{15 \cdot 234} = +0.527$ .  $r_{45 \cdot 123} = +0.087$ .
3. Total radiation  $r_{25 \cdot 134} = -0.410$ .
4. Net assimilation rate  $r_{35 \cdot 121} = -0.382$ .
5. Rel. leaf growth rate

$$R_l = 0.1316T - 0.1085t - 0.3086L + 0.1524R_n - 1.4266 \dots (3).$$

The values of the new partial correlation coefficients have scarcely

<sup>1</sup> W. E. Brenchley: Some Factors in Plant Competition. *Ann. App. Biol.*, vi, pp. 142-70, 1919.

## *on the Growth of Barley.*

changed from those given above, and the surprising result emerges the correlation between net assimilation and relative leaf growth, although, is almost negligible. The experiments on competition by Dr. Bren already quoted, seem to bear out this conclusion, for there large leaf is associated with low net assimilation, and vice versa. Bearing in mind zero correlation means a 'normally' maintained growth rate, it is clear leaf growth is scarcely at all affected by variations in net assimilation. This seems to indicate that under weather conditions such as during early summer in this country net assimilation is maintained at a level that the carbohydrate material formed is always in excess immediate demands of the plant for leaf growth material, and excess be laid down as reserve. It rarely happens apparently that adverse weather conditions last long enough to exhaust these reserves of material for maintaining leaf growth.

Strong evidence for the independence of relative leaf growth rate and net assimilation has also been found by the author in experiments with cucumbers at high temperatures in artificial light, where rapidly falling relative leaf growth rates have been found to accompany steady assimilation rates.

Dr. W. Brenchley's experiments seem also to point in this direction, and it is to be regretted that data of leaf-area were not then taken, as with such additional information the problem of the relation of leaf growth and assimilation rate in her experiment could have been elucidated.

The cause of the harmful effect of high night temperatures on leaf growth must be sought elsewhere. The evidence seems to point to a beneficial effect on the processes of leaf growth and assimilation of large fluctuations, as such, of temperature between day and night, though what this means cannot be formulated in precise terms.

The relative leaf growth rates, calculated from the regression equation (2), together with the values calculated from experimental data, are given in Fig. 4. The agreement in general is good, but is not so satisfactory as that for net assimilation. It must be remembered that, firstly, the 'normal curve' of leaf growth is only approximately known, and, secondly, that sampling errors will play a very large part in determining the results, since we are dealing with differences in slope at points on a curve, and indeed differences between differences.

The correlation coefficients are sufficiently significant when the number of experimental values is taken into account. Moreover, the differences in manuring can have only a small effect on the normal curve, it is unlikely that no influence at all is exerted. /and Ludwig<sup>1</sup> indeed claim to have demonstrated such a change in for the dry weight increase curve.

<sup>1</sup> A. Rippel and O. Ludwig: Untersuchungen über physiologische Gleichgewichtszustände, &c. Biochem. Zeitschr., 1925.

*Gregory.—The Effect of Climatic Conditions*

The consistent variation in level of the calculated and experimental  $r$ s in the curves for 1923 and 1924, as seen in Fig. 4, do in fact point to a common effect. It will be seen, however, that the changes in direction of the curves follow each other closely, indicating that the correlation coefficients are correct in sign, and substantially so in magnitude.

Comparing the curves for experimental values in Figs. 1 and 4, their similarity in form is striking. This similarity is confirmed by taking the partial correlation coefficient of the differences of assimilation and leaf growth from their means. This is found to be + 0.556. If this were the only information on the subject the conclusion that leaf growth and assimilation are highly correlated would be irresistible. The association has been shown to be spurious, and is due to the fact that both processes are correlated positively with day temperature.

In order to test whether humidity plays an important part, as V. Brenchley has suggested, a third set of partial correlations was calculated, in which evaporating power of the air replaced net assimilation, which was known to have little effect. The values are as follows:

Av. day temperature	$r_{15.236} = +0.487$ , significance > 100 : 1	
Av. night temperature	$r_{25.136} = -0.391$ ,	" 24 : 1
Total radiation	$r_{35.236} = -0.422$ ,	" 37 : 1
Relative leaf growth rate	$r_{56.123} = +0.228$ , not significant.	
Evaporating power of the air		

Again the correlations previously established remain almost unchanged, it is seen that the partial correlation with evaporating power is positively large, but not significant. This was quite unanticipated, as it was expected that high humidity would favour leaf growth. Attention has now been called to the unsatisfactory nature of the evaporation data, if they were the only ones available they were perforce used. The found may possibly be due to rainfall causing water-logging of the soil. During rainy weather continual saturation of the soil, by checking the roots, may have acted adversely on leaf growth, so that the positive correlation may mean the beneficial effects of lower water-content of the soil. However this may be, undoubtedly the rate of uptake of nitrogen will influence the leaf growth rate, and this factor has not been taken into account. Heavy rainfall will tend to wash out the nitrate, which would account for the adverse effect found to be associated with high humidity of the atmosphere, and as the drainage water from the pots was replaced as soon as it was cleared, the positive correlation with low humidity would tend to be obscured by this also. (In this connexion see Addendum.)

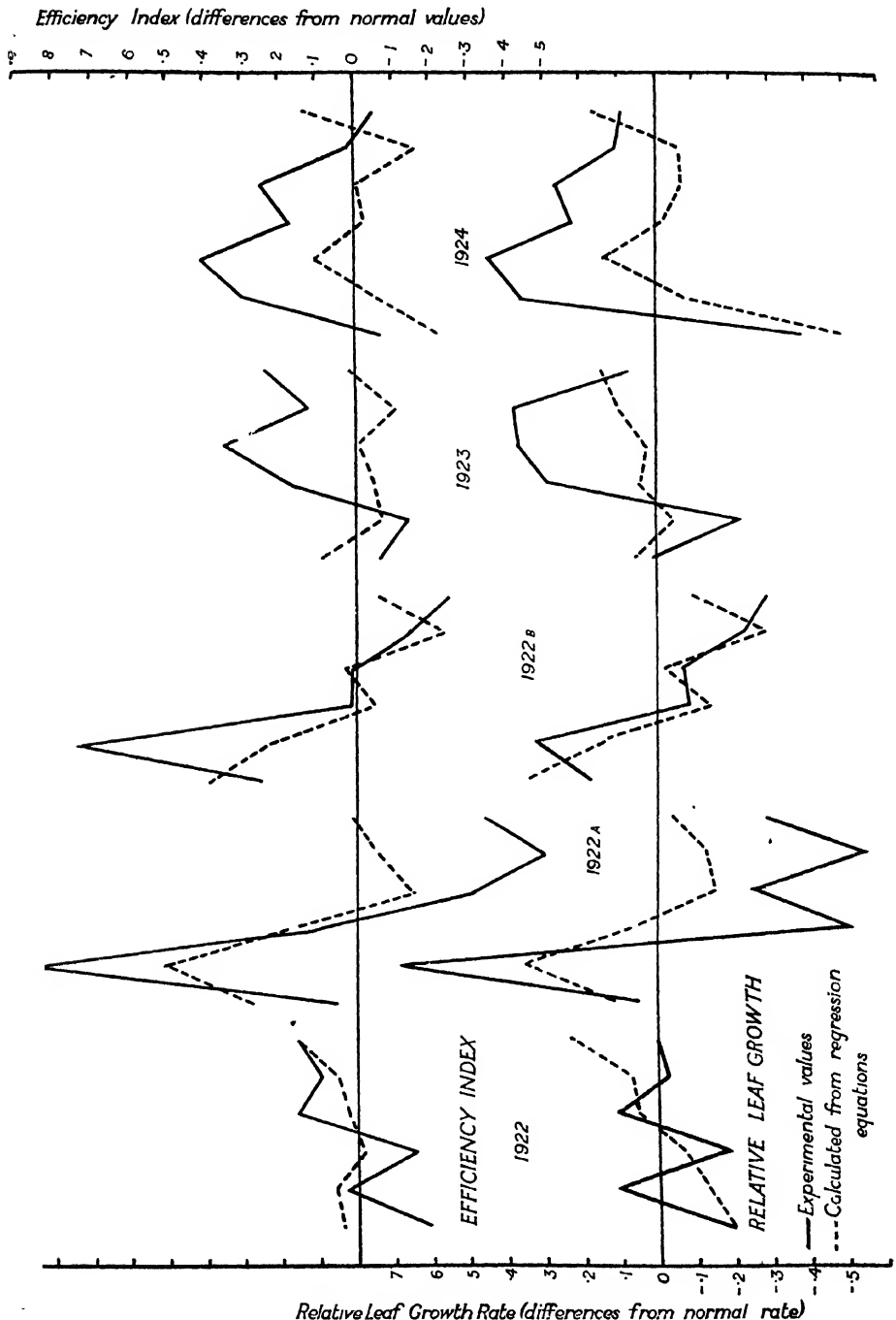


FIG. 4 Comparison of experimental values of efficiency index and relative leaf growth rate with those calculated from regression equations.

Reviewing the information thus far obtained, a clear picture of the adaptation of the barley plant to climatic conditions emerges. The positive and negative correlations of net assimilation and relative leaf growth rates respectively with total radiation provide a mechanism whereby the yield of the plant tends to remain within fairly narrow limits, in spite of climatic variation. Yield depends on total material assimilated, which in turn is determined by two processes, which may by analogy be termed intensity and capacity factors of total assimilation.

The intensity factor is the net assimilation rate which determines the effectiveness with which the leaf surface provides material, while the leaf surface growth, as capacity factor, limits the size of effective area. In a season of high total radiation the negative correlation will condition a low level of leaf growth rate, associated with a high net assimilation, while in a wet season the reverse relations will hold. The product of the two processes will maintain the total of material assimilated within a fairly narrow range. It is, however, necessary to bear in mind that the high temperatures prevailing during bright weather will increase the rate of nitrification in the soil and thus indirectly lead to greater leaf growth by increase of available nitrogen.

#### *The Effect of Climatic Conditions on Efficiency Index.*

The relative rate of dry weight increase is quite analogous with relative leaf growth rate, and may be represented as

$$R_w = \frac{1}{w} \cdot \frac{dw}{dt}.$$

This is the same function as the Efficiency Index of Blackman,<sup>1</sup> and the mean rate is calculated by subtracting the natural logarithms of the weights at beginning and end of a unit time-period.

For the purposes of correlation the whole growth cycle was divided into two parts at the point corresponding with maximum leaf-area.

The two parts were dealt with in different ways, the reason for which will be made clear later.

The 'normal curve' of dry weight increase was obtained by taking geometric means of corresponding values on the standardized experimental curves in a manner similar to that by which the normal leaf-area curve was calculated. The normal curve of dry weight increase is shown in Fig. 3. The early part of the curve is shown separately on a larger scale. The

<sup>1</sup> V. H. Blackman: The Compound Interest Law and Plant Growth. *Ann. Bot.*, xxxiii, 353, 1919.

comparison of experimental efficiency indices and the corresponding 'normal rates' are shown for 1921 in Fig. 5.

The shape of the normal curve of efficiency index need not be discussed here, but it is clear that the growth rate as indicated by the changing slope of the curve is no simple function of time.

If it could be shown that the weight at each moment were related

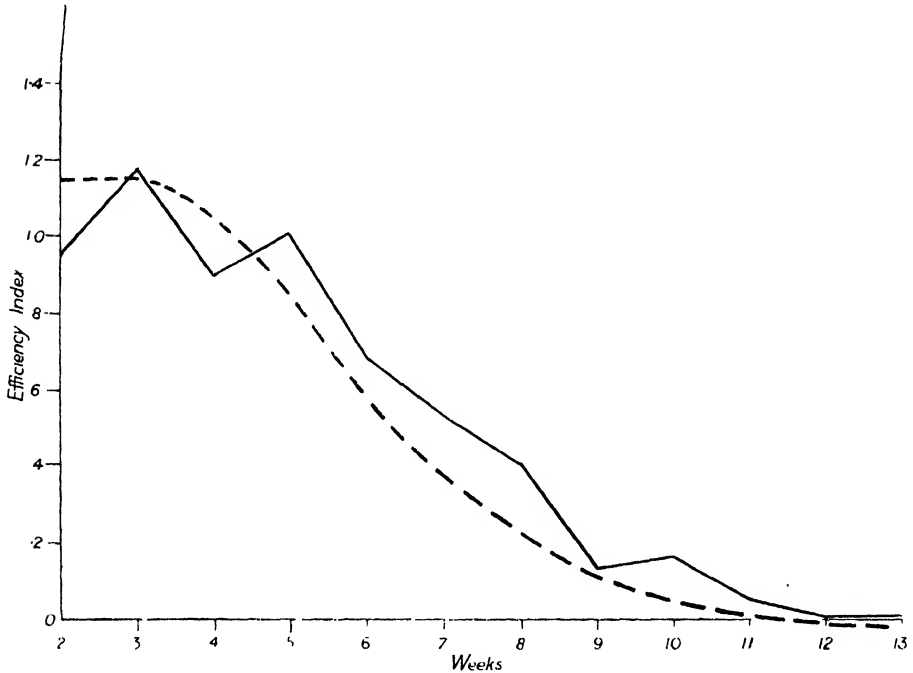


FIG. 5.

in a simple manner to the efficiency index, time as a variable could be eliminated and in its stead the actual weight could be used, and the variation in efficiency index could be stated in terms of percentage of final weight attained. This method of dealing with the problem was suggested to the author by Mr. R. A. Fisher, of Rothamsted Experimental Station. Fig. 6 shows the efficiency index plotted against the percentage of final weight for the series of values of the 'normal curve' (Fig. 3).

It is clear that after 40 per cent. of the final weight has been reached the relation between the variables is strictly linear. The 40 per cent. points corresponds in fact with the point of maximum tillering, and is a critical point in the life-cycle. It is interesting to note that the linear relation of efficiency index and absolute weight is the crucial test for the auto-catalytic nature of the growth process, and Fig. 6 abundantly demonstrates



that only the latter part of the growth cycle can be represented by an autocatalytic reaction equation.

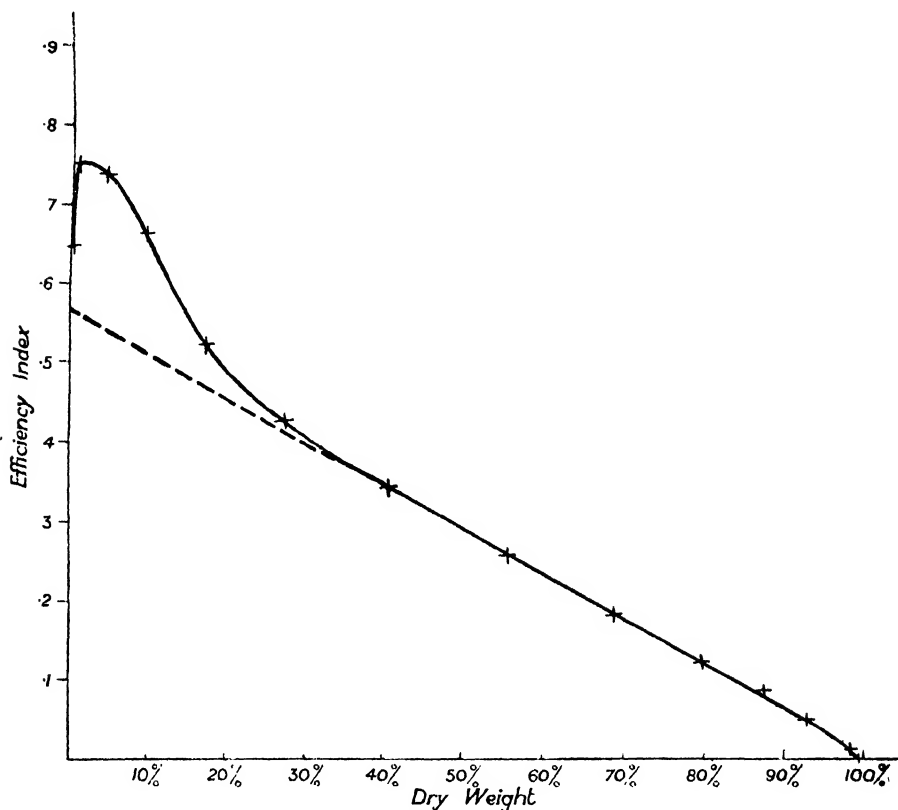


FIG. 6.

*Correlations of Efficiency Index with Climatic Factors in First Part of Growth Cycle.*

The method used was similar to that previously described for relative leaf growth. The factors included were :

1. Average day temperature ( $T$ ).
2. Average night temperature ( $t$ ).
3. Total radiation ( $L$ ).
7. Efficiency index.

The partial correlations and regression equation are presented below :

$$r_{17.23} = +0.441, \text{ significance } 50 : 1$$

$$r_{27.13} = -0.389 \quad \text{ " } \quad 25 : 1$$

$$r_{37.12} = -0.073, \text{ not significant.}$$

$$R_w = 0.1075 T - 0.0999 t - 0.0541 L - 0.797.$$

The only point calling for comment is the partial correlation with total radiation, which is almost negligibly small. This indicates that efficiency index is almost independent of radiation, and that the inverse relations of net assimilation and relative leaf growth with radiation almost exactly balance one another, so that increase in dry weight proceeds at its 'normal rate' whatever the conditions of illumination may be. This, of course, holds true only for the range actually explored, namely, average early summer conditions. The relations of net assimilation and relative leaf growth rate with efficiency index are given by the two following correlation coefficients :

- |                              |                               |
|------------------------------|-------------------------------|
|                              | 1. Av. day temp.              |
|                              | 2. Av. night temp.            |
| $r_{47 \cdot 1235} = +0.646$ | 3. Total radiation.           |
| $r_{57 \cdot 1234} = +0.804$ | 4. Net assimilation rate.     |
|                              | 5. Relative leaf growth rate. |
|                              | 7. Efficiency index.          |

—which show that after allowance has been made for the effect of external factors, efficiency index is strongly correlated with net assimilation and relative leaf growth, and, furthermore, the latter is predominant in effect.

The small correlation of efficiency index with hours bright sunshine found by Dr. W. Brenchley for peas in the early stages of growth ( $-0.0132$ ) may possibly be explained in the same way as the small correlation here found with total radiation. Intense light may in this case also have an inimical effect on relative leaf growth, but the later positive correlation indicates that the detrimental effect is later replaced by a beneficial one. Further investigation of this point is urgently needed, as in this way only can the 'light requirements' of plants be interpreted.

### *Second Part of Growth Cycle.*

The factors included in the analysis are :

1. Average day temperature ( $T$ ).
2. Average night temperature ( $t$ ).
3. Total radiation ( $L$ ).
5. Efficiency index ( $K'_{10}$ ).
8. Dry weight ( $W$ ).

$$\begin{aligned}
 r_{15 \cdot 238} &= +0.388. \\
 r_{2 \cdot 138} &= -0.292. \\
 r_{35 \cdot 128} &= -0.266. \\
 r_{35 \cdot 121} &= -0.942.
 \end{aligned}$$

The linear relation between dry weight and efficiency index is reflected in the very high value of the partial correlation  $r_{85 \cdot 123}$ . The negative sign

indicates the fall in efficiency index as growth approaches its completion. As the ripening process advances assimilation gradually ceases, and this is responsible for the smaller values of the correlations with temperature. The adverse effect of high night temperatures is still apparent. The negative correlation with total radiation has increased considerably, and is easily explained by the observed effect of high light intensity in hastening the dying off of the leaf surface.

The experimental values and those calculated from the regression equation for the first part of the growth cycle are shown in Fig. 4. The general similarity of the curve with the other two already considered is clear, and is due to the high correlation of efficiency index with the other two processes.

From the facts emerging in this analysis the interrelations of the processes determining growth are shown, and the interaction of the climatic complex with the internal factors. This analysis is in the nature of a preliminary survey of the physiological aspects of the problem of the adaptation of the plant to the environment. As suggested in the introductory remarks, the way is indicated towards a true agricultural physiology, which may restate in precise terms much that at present is empirical knowledge. The study of the plant as a whole is needed to test conclusions drawn from laboratory experimentation with single organs where previous history has been assumed to be unimportant, and on single phases of the total life-cycle, whose chief characteristic is an intrinsic unity.

In conclusion, the author wishes to thank Sir John Russell for facilities for experimental work which was carried out at the Rothamsted Experimental Station, Prof. V. H. Blackman for continued interest and inspiration, Mr. R. A. Fisher for invaluable suggestions and patient criticism, and lastly Miss E. D. Kay, without whose co-operation and unflagging industry the necessary routine work of amassing data could not have been completed.

#### SUMMARY.

An analysis of the effect of seven environmental factors on the growth of barley in pot culture has been undertaken.

The environmental factors are :

1. Maximum day temperature.
2. Average day temperature.
3. Minimum night temperature.
4. Average night temperature.
5. Total radiation in calories per sq. cm. per week.
6. Hours bright sunshine.
7. Evaporating power of the air.

Three measures of growth are dealt with :

1. Net assimilation rate (dry weight increase per unit leaf-area per unit time).
2. Relative rate of growth of leaf surface.
3. Relative rate of increase in dry weight (efficiency index).

The whole growth cycle is divided into two parts at the point at which maximum leaf-area occurred, and each half is considered separately.

*Net assimilation rate* during the first part of the growth cycle is shown to be independent of time and of the quantity of nitrogen added as manure. The partial correlations of assimilation rate with day temperature and radiation are significantly positive and high in value, while a significant negative correlation with night temperature obtains. The values of net assimilation rates calculated from the regression equation are graphically presented together with the experimental values, and good agreement is found. Over 80 per cent. of the variation in assimilation rate is accounted for by variation in external factors.

*Relative leaf growth rate and efficiency index.* The method used for the study of the variations in these processes due to environmental changes consists essentially in determining the approximate form of 'normal curves' for the two cases, such that varying rates are conditioned solely by internal factors, while the external factors may be supposed to remain constant at their mean values.

The differences in the slopes of comparable portions of the experimental and 'normal curves' are taken to represent the effects of the external factors, an increase in slope indicating acceleration, and a decrease inhibition. Partial correlations of relative leaf growth rate and average day temperature are significantly positive, and a significant negative correlation with night temperature is again found. A large significant negative correlation with total radiation appears, while relative leaf growth is found to be almost independent of net assimilation rate. A spurious correlation between net assimilation and relative leaf growth is due to both processes being highly positively correlated with average day temperature and negatively with average night temperature.

*Efficiency index* in the first part of the growth cycle is almost independent of radiation, but is highly positively correlated with average day temperature and significantly negatively with night temperature. In the second part of the growth cycle all the correlations decrease in magnitude with the onset of ripening.

The physiological adaptation of the barley plant to climatic changes is brought out clearly by the partial correlations. The antagonistic effects of the correlations with total radiation, *positive* in the case of net assimilation, *negative* in the case of relative leaf growth rate, tend to maintain the yield

constant. In absence of bright sunshine, in so far as the temperature does not fall, the high relative leaf growth rate will lead to a large leaf surface, and hence compensate for the low net assimilation rate, and vice versa. This compensating effect, however, is partially masked by the high nitrification rate in the soil associated with high soil temperature and hence with total radiation; high nitrogen content in the soil will tend to increase leaf growth irrespective of the inhibiting effect of bright sunshine.

#### *Addendum.*

Since the foregoing was written the analysis has been carried a step farther, and the influence of the nitrogen factor in growth has been investigated. It has been shown that the discrepancies in relative leaf growth rate between the experimental values and those calculated from the regression equation (shown graphically in Fig. 4) can largely be accounted for by the nitrogen relations. The residual values for relative leaf growth rate, namely, the differences unaccounted for by the variations in external factors, have been correlated with the departures from the mean values at the times of sampling of the nitrogen content of the leaves, expressed as percentage nitrogen in the dry weight. The correlation coefficient is  $+0.635$  (significance  $> 100:1$ ). It thus appears that the consistent difference of level between experimental and calculated values for 1923 and 1924 is due to a consistent high value, during these two periods, of the nitrogen content of the leaves. As this figure for nitrogen content is independent of leaf-area there can be no question here of a spurious correlation arising from an indirect correction for leaf-area discrepancy.

The correlation between the same residuals and the ratio of weight of nitrogen in the leaves to the weight of nitrogen in the remainder of the plant is found to be  $+0.632$ , which is almost identical with the correlation value already given. As here the leaf-area is indirectly involved, since the total weight of nitrogen in the leaves depends on the total leaf surface, this correlation standing alone might be attributed to a spurious relation. In view, however, of the identity of the two values it seems probable that the excess nitrogen content of the leaf is accompanied by a corresponding high nitrogen content of the rest of the plant.

Evidently the discrepancies observed in Fig. 4 are not fortuitous, but indicate that relative leaf growth rate is largely dependent on internal factors and is relatively independent of external conditions; whereas, in contrast with this, net assimilation rate has been shown to be wholly controlled by external factors.

# The Changes induced in the Anatomical Structure of *Vicia Faba* by the Absence of Boron from the Nutrient Solution.

BY

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*Rothamsted Experimental Station.*

With nine Figures in the Text.

## INTRODUCTION.

THE chemical elements essential for the healthy growth of plants are now recognized to be more numerous than was originally supposed, and although some must be supplied in considerable quantity, of others only a trace is required. Boron is an example of the latter type of element. Experiments at Rothamsted have shown that *Vicia Faba* (broad bean) and several other leguminous plants fail to complete their development in water or sand culture unless a trace of this element is supplied. 1 part  $H_3BO_3$  in 25,000–12,500,000 parts of nutrient solution being sufficient (1, 4). In its absence characteristic symptoms are exhibited in both shoot and root. In the case of the broad bean the flower buds shrivel and fall off and the stem withers and blackens at the apex, the injury gradually travelling down the plant; the root system is stunted, the laterals being few in number and often thickened. A comparison between the anatomical structure of such abnormal shoots and roots with those of the healthy plant is the subject of the present paper.

## METHODS.

The material examined was obtained from broad beans grown during 1921–4, at various times of the year, in one of two 'complete' nutrient solutions. The latter were fundamentally of the same composition, but in the one the phosphate was supplied wholly as  $KH_2PO_4$ , giving a pH value of 3.8, and in the other as a combination of  $K_2HPO_4$  and  $KH_2PO_4$ , with a

pH value of 6.2. Although both allowed of satisfactory growth, the less acid medium proved the more favourable of the two. However, as regards structural features, the plants grown in either solution were indistinguishable, so that all the material has been considered together. The sets of plants for comparison, whether supplied with boron ( $H_3BO_3$  1 : 5,000–1 : 2,500,000) or not, were in every case started simultaneously and grown under identical conditions. The material from each series was pickled when the characteristic withering and discoloration were apparent in the shoots of those deprived of boron. None of the sections described, therefore, is from very young plants, since the 'dying off' does not set in until after four or more weeks' growth in the nutrient solution, i. e. when the healthy plant is about to flower. This initial normal development in plants not supplied with boron has been attributed to the boron in the seed, the presence of which can be readily detected.

The fixative employed at first was one part glacial acetic acid and three parts 95 per cent. alcohol; although fairly satisfactory a warmed mixture of 3 grm. corrosive sublimate, 3 c.c. glacial acetic acid, and 100 c.c. 70 per cent. alcohol was eventually used, as less shrinkage of the tissues was incurred. In the latter case, the material was finally washed in successive changes of 70 per cent. alcohol until the brown colour caused by the addition of a drop of iodine solution was retained. Dowson's quick method of paraffin infiltration was employed (2), viz. the material after dehydration was transferred directly to a mixture of paraffin (M.P.  $52^{\circ}$  C.), xylol, and absolute alcohol in the proportions 1, 2, 3 respectively, and placed corked inside the oven. After twenty-four hours the xylol and absolute alcohol were allowed to evaporate off and the material then embedded in fresh wax.

Sections were cut at  $10\mu$  and stained with a combination of gentian violet (sat. sol. in 40 per cent. alc.) and vesuvian brown (sat. sol. in 50 per cent. alc.). The slide was immersed in the gentian violet for ten minutes, transferred without previous washing to the vesuvian brown for a few seconds, and then rapidly passed through a 50 per cent. mixture of xylol and absolute alcohol before clearing in xylol and mounting. The differentiation proved entirely satisfactory, the lignified tissue and endodermis in the root retaining the violet stain, the other tissues taking the brown colour. This method had the special advantage of allowing a large number of preparations to be completed in a very short time.

Since all the material had been obtained from plants grown in water culture it was necessary to ascertain whether their anatomical structure was similar to that of broad beans grown under ordinary cultural conditions in soil or had been in some way modified. Several sections of stems from broad bean plants grown in soil (pot culture) were, therefore, examined for the sake of comparison.<sup>1</sup>

<sup>1</sup> For these I am indebted to Dr. J. Davidson.

## I. ANATOMICAL STRUCTURE OF THE STEM.

A. *Normal.*(1) *Plants grown in Soil.*

In transverse section approximately 6-10 small vascular bundles lie along each side of the square stem, a larger bundle occurring at each corner. The cortex is narrow and composed of thin-walled parenchymatous tissue. The pith also consists of thin-walled cells, which eventually break down with the formation of a central cavity. The structure of the vascular bundles is that of a typical Dicotyledon. Large unthickened cells, lying between the tracheides and the cambium, are a prominent feature before much secondary thickening has taken place. These appear to be as yet non-lignified xylem elements, the presence of such tissue being in keeping with the rapid growth and succulent nature of the plant. Later, however, all the xylem becomes thickened in the usual manner, and groups of sclerized tissue are developed on the outside of the phloem. A typical, regular, small-celled cambium is always clearly defined; the importance of this statement will be evident when the stem structure of plants deprived of boron has been described.

(2) *Plants grown in Water Culture with Boric Acid.*

As regards external appearances these plants are perfectly normal (Fig. 1). The structure of the stem appears to be essentially the same, whether the quantity of boric acid supplied is beneficial, e.g. 1 : 2,500,000, or slightly toxic, e.g. 1 : 5,000, and sections from either set are indistinguishable from those of soil-grown plants as described above. These water-culture plants, therefore, provide a standard of normal anatomical structure for the broad bean, with which comparisons may justly be drawn.

B. *Abnormal.**Plants grown in Water Culture without Boric Acid.*

Transverse sections of the stems of plants which have already begun to wither and blacken at the apex in the characteristic manner exhibit certain distinct abnormalities of structure. The vascular bundles in particular are affected, the xylem often appearing unusually remote from the phloem or even broken up into small groups of elements. The resulting irregularity affords a striking contrast to the almost diagrammatic form of the vascular system in the healthy plant. Patches of blackened and disintegrated tissue also occur, which are particularly associated with the vascular bundles.



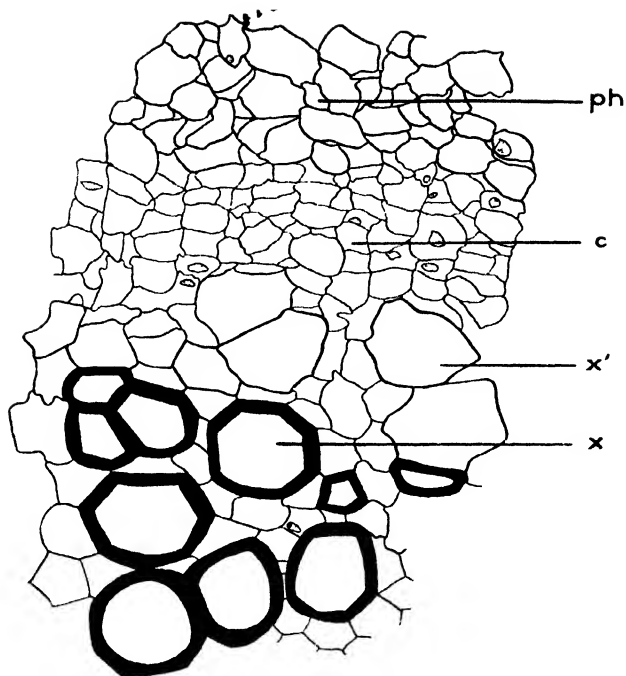


FIG. 1.

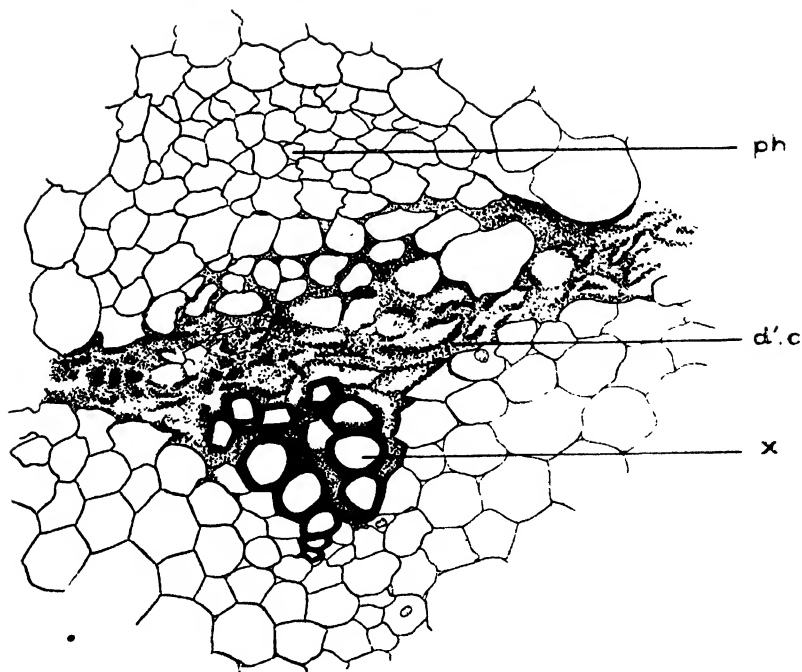


FIG. 2.

FIGS. 1-4. Transverse section. Vascular bundles near apex of stem of *Vicia faba* grown in nutrient solution. *c.* = cambium; *ph.* = phloem; *x.* = lignified xylem.  $\times 333$ .

Fig. 1. Grown with boron; normal structure. *x'*. = unthickened xylem.

Fig. 2. Grown without boron, showing *e.c.* = enlargement of cambial cells; *d.c.* = initial de-

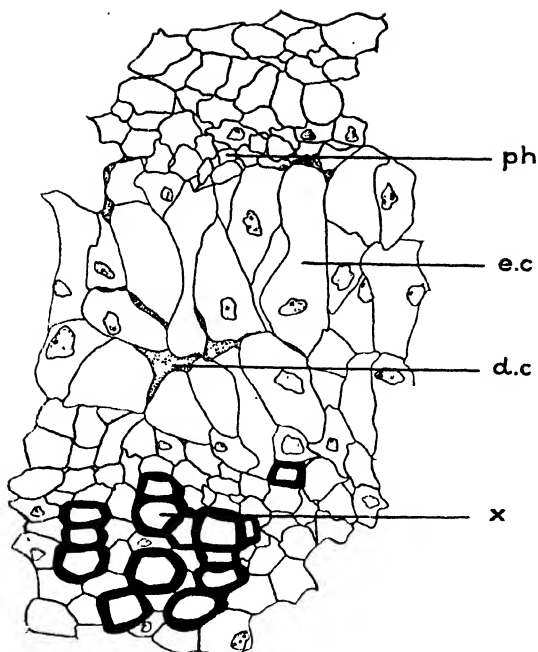


FIG. 2.

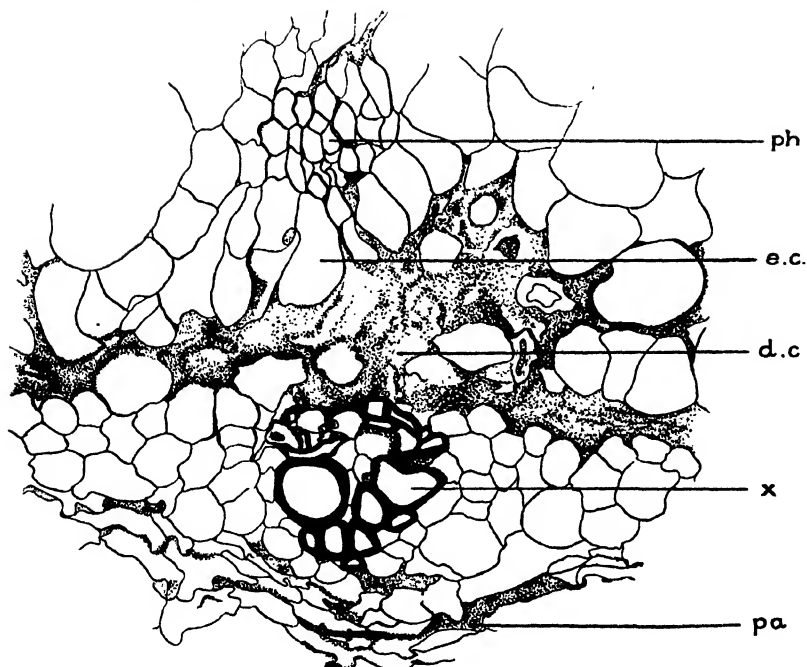


FIG. 4.

Fig. 3. Grown without boron, showing *d.c.* = disintegration of cambium without previous enlargement.

Fig. 4. Grown without boron, showing *d.c.* = disintegration of previously enlarged cambium (*e.c.*); *pa.* = disintegration of ground parenchyma.

On closer inspection it is evident that an unusual development of the cambium is chiefly responsible for this abnormal appearance, although other tissues may frequently be involved.

*Types of Abnormality in the Cambium.*

In the cambial layer two principal types of abnormal structure occur.

1. *Enlargement of Cells.*—In many cases large, elongated cells with prominent nuclei occupy the position of the usual small-celled fascicular and interfascicular cambium (Fig. 2). Although the elongation tends to be in a radial direction, the development may be quite irregular, and in longitudinal section appears to follow no rule at all. Such cells may be associated either with young vascular bundles or with those showing quite advanced secondary thickening. In the former case, the xylem and phloem are situated in their usual position during the early stages of this abnormal growth, but later the elongating cells may break up the xylem elements into small groups, the phloem also becoming compressed or displaced. As would be expected, however, actual displacement of the xylem and phloem is not evident in bundles where much secondary thickening has taken place, i. e. in the older parts of the stem, which do not suffer injury until some considerable time after the young apical tissues.

2. *Disintegration.*—In other cases the cambium is more or less completely broken down, a mass of brown or black tissue being found in its place (Fig. 3). The unthickened phloem elements may possibly also participate in this disintegration, which, however, originates in the cambium. These atrophied cells account for the black streaks in the stem, which to the naked eye in transverse section appear to follow the course of the vascular strands. The possibility of this disintegration being due to bacterial infection has been considered, but examination<sup>1</sup> of some of the most severely affected material provided no evidence that this was the case.

The above two phases of cambial affection may occur together in the same part of the stem (Fig. 4). On the other hand, the disintegration may be independent of any definite elongation, although there is usually indication of some abnormal condition in the cambial region, where the degeneration is less well established. Similarly, if the enlargement of the cambium is the principal feature, at least traces of cell-wall disintegration are always apparent either in the same or a neighbouring tissue (Fig. 2).

It seems, therefore, that elongation of the cambium is frequently the first apparent effect of boron deficiency. This is borne out by the fact that it was the chief abnormal feature in the anatomical structure of (1) the apical portion of a stem which externally exhibited only the early symptoms of a lack of boron, viz. the leaves and growing-point were

<sup>1</sup> For which I am indebted to Mr. H. G. Thornton.

unhealthy, but there was no actual withering or discoloration, and (2) the central portion of a stem which still retained a normal appearance, although its apex was considerably withered.

Actual disintegration of the cambium usually arises later in the course of disorganization, although it may set in quite early before any elongation of the cells has occurred. The time required for this degenerate stage to be attained is presumably determined by (a) the rate of growth, itself dependent on seasonal conditions, (b) the amount of boron originally present in the seed, (c) the individual vigour of the plant, and possibly other factors as well. The nature of the cambial disorganization to be found would, therefore, depend on these phenomena, i.e. upon the rate at which the deficiency factor was affecting the plant. The occurrence of both types of abnormal cambium, either alone or in combination, would thus be readily accounted for. The possibility of the one kind of injury being correlated with the other will be discussed later.

At any particular part of the stem, all the vascular bundles do not necessarily show the same degree of disorganization, those at the corners being often the most severely affected; an instance may also be cited where, at the same level, the bundles on one side of the stem were quite normal in structure, and showed well-developed secondary thickening, whereas those on the other side were but incipient, badly distorted, and in some cases considerably discoloured, a few being of an intermediate type.

Besides this injury in the cambium, certain other abnormal features in the structure of the stem are frequently found.

(a) The pith and ground parenchyma, particularly that in association with the xylem, often show a cellular disorganization or disintegration which results in patches of blackened tissue, the cell-wall apparently being primarily affected (Fig. 4).

(b) The large thin-walled xylem elements, which are such a prominent feature at the apex of the stem in healthy boron-treated plants, are invariably absent. This may be correlated with the arrested growth and impaired vitality of the plant, for young tissues or those of a succulent nature would hardly be expected to occur under such conditions (cf. Figs. 1 and 2).

(c) The xylem itself may degenerate with the formation of a discoloured mass.

(d) The lumen of the tracheides is relatively small, and frequently more or less completely blocked by some unknown substance.

That the anatomical structure as well as the external appearance of these plants in the early stages is suggested by the occurrence of a variety in the degree of injury exhibited, ranging from a nearly normal condition to one of great disorganization. This is corroborated by the initial healthy structure of the lateral shoots, to which reference is made later.

In making a comparison, however, between the anatomical structure of plants grown with and without boric acid, it is necessary to bear in mind that apparently similar parts of the two sets of plants are not strictly comparable. For instance, whereas the apex of the healthy shoot is only of recent development and retains considerable meristematic activity, that of the withered plant has already been in an arrested condition of growth for some time, and is therefore appreciably older. This would account for the occurrence near the apex of the dying shoot of secondarily thickened vascular bundles, a condition usually associated only with older parts of the stem, and, further, might afford additional explanation, if required, of the absence of thin-walled xylem elements, as all the tracheides would be already thickened; however, it is equally probable that the latter have been involved in the disintegration process of the adjacent cambial tissue, wherever this has occurred.

It is frequently noticeable that broad beans grown in water culture without boron, after showing the characteristic apical withering of the main stem, develop new lateral shoots. These behave in exactly the same manner as the main axis, viz. they grow normally for some time, but later begin to wither, starting at the growing-point, and fail to flower. On the other hand, any lateral shoots produced by boron-treated plants always remain entirely healthy. Sections were, therefore, cut from two of these apparently healthy lateral branches of plants suffering from a deficiency of boron, and comparison made with the structure of their respective withering main shoots, to see (1) if the external appearance was any criterion as to the condition of the internal structure, and (2) if it was possible for one part of the plant to be healthy and another part definitely suffering from a lack of boron.

Whereas both the dying main stems showed an abnormal structure as has just been described, neither of the lateral shoots were similarly affected, both being practically normal at this stage. One, however, showed indications of incipient cell-wall degeneration in parts of the cambium and phloem, and it is noteworthy that of the two this shoot appeared to be slightly less healthy than the other. Such lateral branches ultimately evince an abnormal structure similar to that of the main stem. As would be expected, the structure of lateral shoots of boron-treated plants remains perfectly normal throughout growth.

Thus it would seem that there is a close correlation between the external appearance and internal structure of the stem, and that in the event of a deficiency of boron arising an abnormal condition is induced in both cases. This deficiency, therefore, at first causes local injury only, which appears primarily at the chief growing apex, and does not in any way prevent the development of new, and in the young stages healthy, secondary shoots from the base of the stem; in fact, plants grown without boron show

a tendency to produce an unusually large number of lateral shoots. This is probably analogous to the common inducement of lateral branching in both shoot and root by injury to the growing apex of the main axis. However, since each of the lateral shoot apices later become affected in the same manner as the main axis, it would seem that the field in which the deficiency factor is operative gradually becomes enlarged until the whole plant is more or less completely affected.

## II. ANATOMICAL STRUCTURE OF THE ROOT.

The correlation between the internal structure and external appearance in the root is less close than in the stem, a fact which greatly increases the difficulty of selecting suitable material for anatomical examination. For example, the root system of a plant grown without boron may be characteristically stunted and thickened and show the usual lack of tertiary roots, yet many of the individual branches exhibit no abnormality in structure. This is all the more unexpected since most of the material was pickled when the apex of the shoot showed definite signs of withering, which does not occur until some little time *after* the peculiar type of root system can be recognized. It would seem, therefore, that growth of the roots is in an arrested condition for a considerable period before any abnormal structural developments occur. However, actual blackening of the root-tip, which is not seen until the shoot is appreciably withered, is invariably accompanied by structural disorganization. It is noteworthy that although the anatomy of both the root and shoot of plants deprived of boron may be quite normal at certain stages, no case has occurred where a boron-treated plant exhibited any of the structural peculiarities here attributed to a deficiency of that element.

As has been already stated, the material was obtained from plants grown in water culture. Since the nutrient solution was not inoculated, no nodules formed on the roots, the necessary nitrogen being supplied as usual in the form of nitrate. The structure and development of the nodule of the broad bean in the presence and absence of boron have been described elsewhere by Brenchley and Thornton (1).

### A. *Normal.*

#### *Plants grown in Water Culture with Boric Acid.*

These roots were long, fine, and freely branched, as is characteristic of all healthy boron-treated plants. Sections were cut of roots of various ages and at different parts of the same root ; in every case the structure proved entirely normal (Fig. 5). The majority are either triarch or tetrarch, though a few may be pentarch or hexarch. Secondary thickening results in the

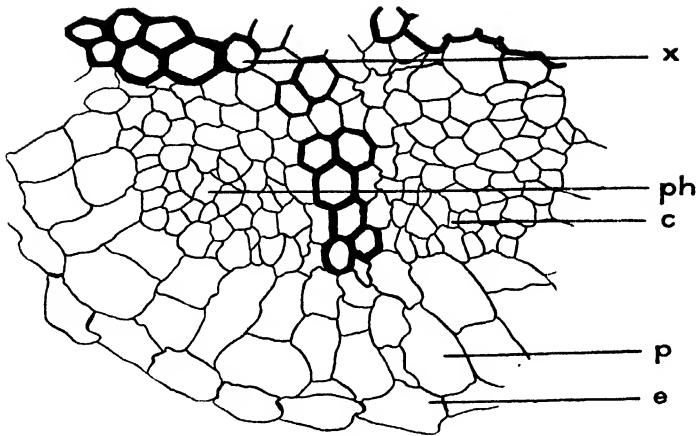


FIG. 5.

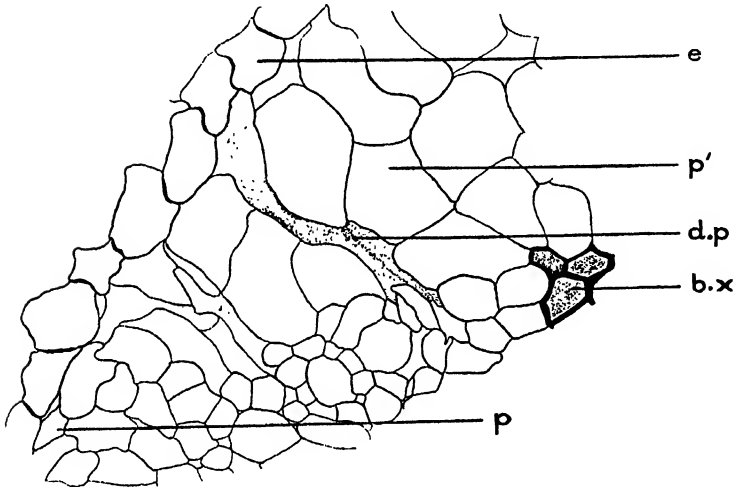


FIG. 8.

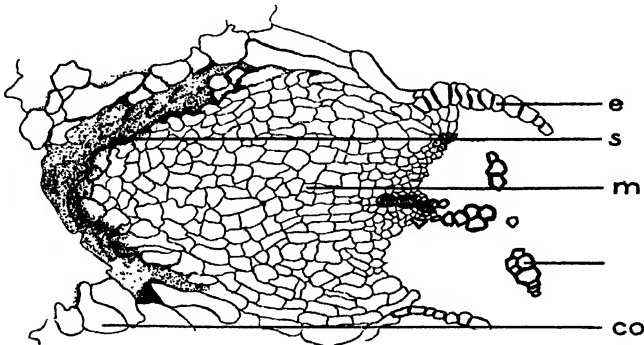


FIG. 9.

FIGS. 5-9. Transverse section. Part of root of *Vicia Faba* grown in nutrient solution. *c.* = cambium; *ph.* phloem; *x.* = lignified xylem; *p.* = pericycle; *e.* = endodermis; *co.* = cortex.  $\times 333$ .

Fig. 5. Young root grown with boron; normal structure.

Fig. 6. Grown without boron, showing early stage of abnormal development. *d.c.* = degeneration of cambial cells; *b.x.* = partially blocked tracheides; *d.ph.* = disorganized phloem; *d.x.* = disorganized xylem.

Fig. 7. Older root grown without boron, showing later stage of abnormal development.

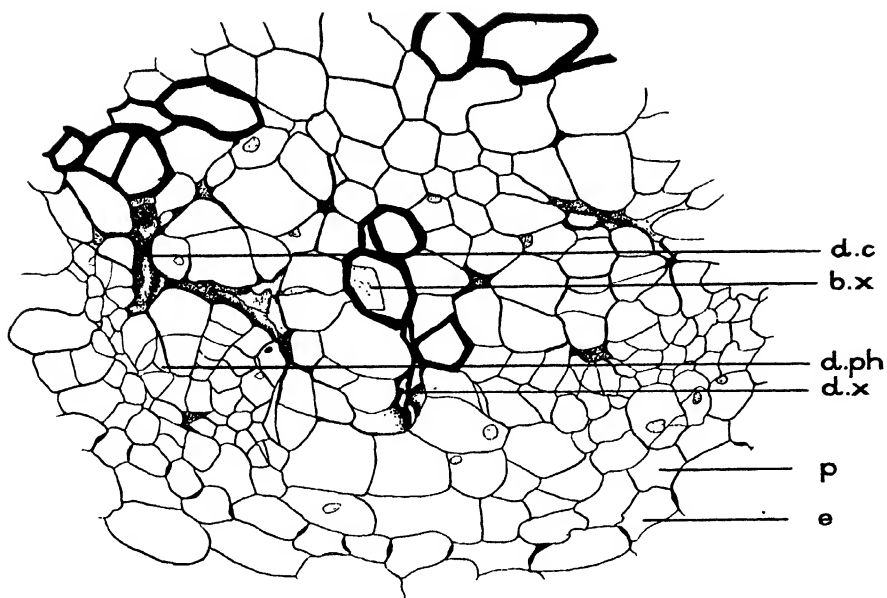


FIG. 6.

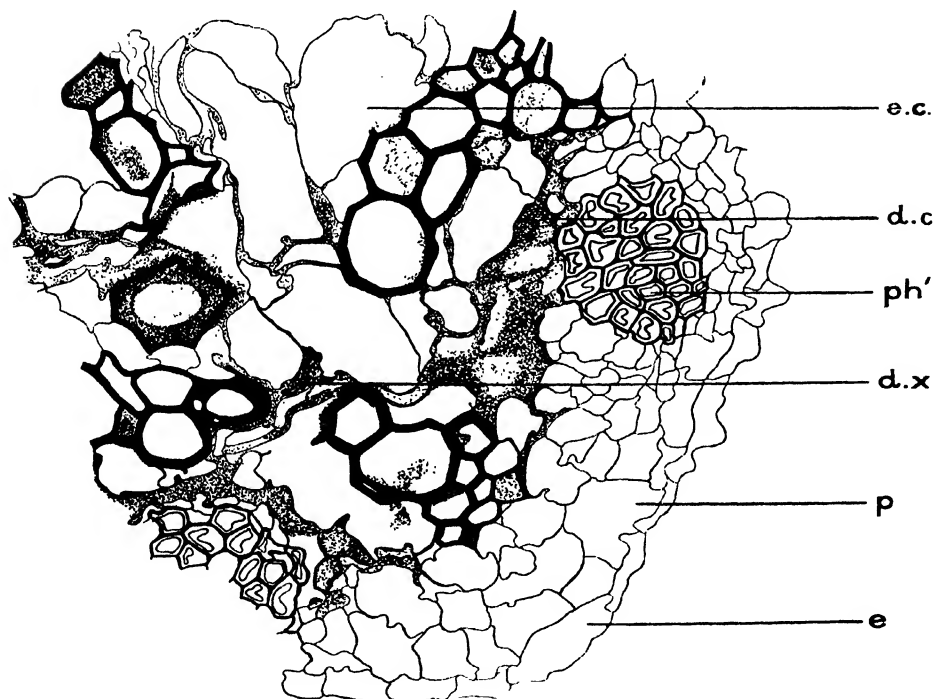


FIG. 7.

*e.c.* = enlarged cambial cells; *d.c.* = disintegration of cambium; *ph'* = phloem fibres; *d.x.* = degenerating xylem.

Fig. 8. Grown without boron, showing irregular development of pericycle. *p'* = enlarged cells of pericycle; *p.* = small-celled pericycle; *d.p.* = initial degeneration of pericycle; *b.x.* = blocked tracheides.

Fig. 9. Grown without boron, showing check to development of young rootlet (diagrammatic; outline only drawn with camera lucida). *s.* = blackened sheath surrounding apex of rootlet; *m.* = meristematic tissue.  $\times 93$ .



formation of a central core of tracheides and sclerized tissue, and other small masses of sclerenchyma occur on the outside of each phloem group. A normal active cambium is universally present, and the pericycle, consisting of one or two layers, is bounded by a well-defined endodermis.

### B. *Abnormal.*

#### *Plants grown in Water Culture without Boric Acid.*

Although all the roots examined were obtained from plants whose shoot apices were showing definite signs of a deficiency of boron, their anatomical structure was very varied. In some cases the stele was either completely disorganized or showed definite peculiarities of structure, whereas in others the roots were quite normal, or at most exhibited merely a general unhealthy appearance. The latter was particularly the case in the older parts of roots where considerable secondary thickening had taken place. This gradation in the degree of injury would seem to justify the conclusion that, as in the stem, the root structure is perfectly normal for a certain length of time, the irregular condition arising later, presumably when the deficiency factor becomes active in that part of the plant. In the early stages therefore the injury is localized, as otherwise the occurrence of both healthy and abnormal roots on a plant which is clearly suffering from lack of boron, as implied by the appearance of the shoot, could not be accounted for.

This variety in the root structure had been partly anticipated owing to the extensive branching of the primary root of the broad bean. Since, as has been described above in the case of the stem, it was possible for the comparatively limited number of lateral shoots of plants suffering from a deficiency of boron to differ in structure both from the main axis and each other, it seemed probable that under the same circumstances a similar state of affairs would occur to an even greater extent in the root system, owing to the larger number of branches, differing widely as to age, size, and order, which are necessarily involved.

The principal abnormal feature of these roots is their stelar structure, or if the part of the root in question is too young for this to be fully differentiated, the tissue destined to give rise to the stele. In the latter case older parts of the same root invariably show an abnormal vascular structure. The chief peculiarities are as follows:

(i) The thin-walled tissues, e. g. cambium, phloem, and ground parenchyma, are apparently first affected (Fig. 6). The cells lose their firm outline and brown streaks are noticeable between them. Their development is irregular, a few rather large cells often occurring in place of a number of smaller ones. This is particularly noticeable in the region of the cambium, and it is probable that this effect is partly due to the breaking down of

some individual cells rather than to any enlargement of existing ones (Fig. 7). There is no indication, however, of these cells attaining either the form or size of those of the stem cambium. The brown discoloration is presumably the initial stage in the disintegration process which all these unthickened tissues undergo in the severely affected roots (Fig. 7).

(ii) In some cases the pericycle is abnormal (Fig. 8). The cell divisions take place irregularly, accompanied by discoloration of the walls, or the cells may be abnormally large, being elongated in a radial direction. The irregular development of the pericycle may possibly be regarded as due to the suppressed development of root initials, which, owing to the injury sustained by meristematic tissues from the lack of boron, are unable to develop properly. This explanation receives some support from (iii).

(iii) The young rootlets fail to penetrate the cortex, or cease developing soon after their emergence. This readily accounts for the characteristic lack of tertiary rootlets in all plants grown without boron. A sheath of blackened tissue is invariably found enveloping the tip of such rootlets (Fig. 9), a fact of particular interest, since similar sheaths are described by Brenchley and Thornton (1) as surrounding the abortive root nodules of broad beans grown in inoculated water or sand culture without boron. The actual nature of the sheath is unknown, but from its appearance and black or brown colour would seem to result from the atrophy of some tissue.

The failure of these roots to emerge may be regarded as an advanced condition of (ii), the sole difference being that in the latter case the deficiency factor comes into force at an earlier date in the development of the new root. As regards the thickened and stunted external appearance of the roots, probably both (ii) and (iii) are responsible, although the absence of tertiary rootlets, which would tend to obscure the older and larger branches, may itself accentuate the effect.

(iv) The xylem is usually poorly developed, and although it stands out well in sections where the injury is but slight, it suffers disintegration in badly affected roots. Blocking of the lumen of the tracheides is also of frequent occurrence (Fig. 7).

Reference has already been made (p. 38) to the abnormal condition of young undifferentiated stelar tissue in the root-tips of plants grown without boron. This was seen to be of two types, the one merging into the other as the older portion of the root was reached; (a) at the extreme apex the cell contents were blackened but the walls though discoloured retained their outline, (b) just behind the apex the cell walls were both discoloured and broken down, but in every instance a connexion could be traced with an abnormal stele in the still older parts of the root, and the pericycle was often abnormal even at this stage. Some discoloration, however, occurred occasionally at the root-tip of boron-treated plants, but in the latter cases it was found to be of the (a) type only, and moreover the vascular structure

proved to be entirely normal, showing that discoloration of this nature does not necessarily indicate or result from a lack of boron.

Although it is chiefly the stelar structure that is affected by a lack of boron, the cortex may also be involved, blackening or atrophy of the tissue, particularly of the (a) type, being frequently found. However, as in the case of the root-tips, discoloration of the cortex should not necessarily be attributed to a lack of boron unless accompanied by the more reliable criteria of peculiarities in the vascular structure, since forms of cortical injury are occasionally found in plants treated with boron.

Taken as a whole, therefore, the anatomical structure of both shoot and root is affected by a lack of boron in much the same way, the points of similarity being :

(a) The prevention of the normal development of meristematic tissues as seen by—

(i) Death of the growing apices in each case. Failure of young roots to emerge or complete their development.

(ii) Withering of lateral shoots in a manner similar to the main axis.

(iii) Abnormal form and eventual destruction of the cambium in both cases.

(b) Poor development of the xylem, and in the later stages degeneration with discoloration of the xylem, phloem, and ground parenchyma.

#### DISCUSSION.

That boron plays an all-important part in the metabolism of certain plants is clear, since death ensues in its absence, but whether its action is direct or indirect has not yet been determined. Any theory, therefore, that attempts to explain the abnormal anatomical developments which arise if it is withheld must at present be accepted with due reservation.

The fact that boron can be detected in the stem, leaves, and pods of the broad bean implies that the element becomes distributed throughout the plant after absorption ; and, further, the need for the supply of boron to be maintained during the life of the plant indicates that the initial reserve of the element in the seed is insufficient for the needs of the plant, and that it is in some way fixed and not in a state of circulation (4). It would seem, therefore, that death of the apical meristem, which occurs when boron is withheld, results from the lack of some factor essential to growth (or at least to the development of permanent from meristematic tissue), which is itself in some way dependent either directly or indirectly on the presence of this element.

Although it is possible that boron exerts a direct influence on the development of the meristematic tissues, both apical and cambial, it seems probable that secondary reactions are also concerned, such as are tentatively suggested below.

Priestley and Woffenden (3) have demonstrated that the blocking of a parenchymatous surface and the subsequent accumulation of sap are essential antecedents to meristem formation, thereby explaining the endogenous development of lateral roots and the activity of a phellogen in cork formation.

The undifferentiated apical meristem of the shoot approximates to such a parenchymatous tissue. Since this is the region where injury from a lack of boron is first evinced it seems justifiable to conclude that it is the seat of the primary disorganization of the normal functions of the plant, and that when rendered inactive by such injury, it might simulate a blocked parenchymatous surface. As food supplies are directed chiefly to those parts of the plant where growth is most vigorous, e. g. apices of shoot and root, there is reason to suppose that sap might accumulate behind this withering apex. The occurrence of blocked tracheides affords support to this theory, as such a condition of affairs would be readily correlated with an inhibited circulation of sap. In this way the two essential conditions for the activity of a meristem indicated by Priestley and Woffenden (3) would be fulfilled.

Intimately connected with the apical meristem are the fascicular and interfascicular meristems (i. e. cambium), which also suffer ultimate degeneration, and it would seem legitimate to suppose that this injury is due to the absence of boron, as is the case in the growing apex. Since the withering gradually travels down the plant, the cambial tissue first to suffer is that situated close behind the now withering apex where conditions suitable for meristematic activity may prevail. It is possible that the hypertrophy of the individual cells of the cambium in this position is the primary result of the lack of boron acting on meristematic cells so situated. No evidence is available that any mechanical injury to the growing apex causes similar hypertrophy, indicating that where the latter occurs some physiological factor, such as that supplied by a lack of boron, is at work. The eventual death of this hypertrophied cambial tissue would itself provide a blocked surface capable of accounting for the similar enlargement of the cambium in the lower and as yet apparently normal parts of the stem, provided of course that the deficiency factor was still in force. Under certain circumstances, presumably when the deficiency factor is particularly acute, death of the cambium occurs without previous enlargement of the individual cells, thus accounting for the second type of abnormal cambium.

This theory may also be applied to the root, where under the same conditions a somewhat similar abnormal structure occurs, the early degeneration of the cambium being ascribed to the direct influence of the deficiency factor, and the hypertrophy to its situation in a region conducive to meristematic activity when suffering from a lack of boron. However, as has already been stated, the enlargement of the cambial cells is distinctly less

evident in the root than in the stem. This may be possibly due either to (a) the presence of an endodermis in the former, which by controlling the sap circulation or by some mechanical means might inhibit the expansion of the affected tissue, or (b) the absence of actual withering of the apical meristem of the root, owing to its immersion in a liquid which might prevent the formation of a proper blocking surface.

It must be emphasized, however, that this theory has been brought forward in an attempt to account for the abnormal structure of broad bean plants suffering from lack of boron, and although the facts may seem to be well established, the explanation of them is by no means incontrovertible. Further work alone will show whether or not such a hypothesis is justifiable.

#### SUMMARY.

(1) The anatomical structure of the stem of *Vicia Faba* grown in a nutrient solution containing a small quantity of boric acid (e.g. 1 : 2,500,000) is normal, being exactly similar to that of plants grown in soil.

(2) If boron is omitted from the nutrient solution the structure of both the stem and root becomes abnormal, the chief features being :

(a) Hypertrophy of the cells of the cambium followed by degeneration with discoloration, or direct disintegration of the same tissue without previous enlargement.

(b) Frequent disintegration of phloem and ground parenchyma.

(c) Poor development of xylem and in some cases ultimate breaking down of this tissue.

(3) A definite connexion exists between the presence or absence of boron and the anatomical structure, and the correlation of this with the meristematic activity of the plant is discussed.

In conclusion, I wish to thank Dr. W. E. Brenchley for her valuable advice and criticism throughout this investigation.

---

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## The Endophytic Fungus of *Lolium*.

### II.<sup>1</sup> The Mycorrhiza on the Roots of *Lolium temulentum*, L., with a Discussion on the Physiological Relationships of the Organism concerned.

BY

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With Plates I-III.

#### INTRODUCTION.

IN a previous paper (22) an attempt was made to add to our knowledge concerning the fungus associated with different species of the genus *Lolium*; that contribution dealt mainly with the embryology and mature structure of the grain, and the relation of the fungus to the plant during these varied stages. At that time I had not made any exhaustive examination of the root-system, and it was, and is, generally thought that the roots of these grasses, are devoid of any fungal association. Since the publication of Part I of this paper I have restricted my attention to the roots of the *Lolium* grasses and have been able to demonstrate the presence in them of a typical endophytic mycorrhiza, an account of which is given in the following pages.

It will not be necessary to attempt an historical review of the work done on mycorrhizas up to the present time, since the literature on this subject is very extensive and has been ably reviewed by many workers; the well-known papers of Jænse (11), Gallaud (9), and Bernard (2) include very comprehensive historical accounts of the literature carrying us to 1909. Since that date there have been many additions to these lists, of the more recent the most interesting being Kusano (15), working with *Gastrodia*, and Rayner (26, 27, 28, and 29) with the *Ericaceae*, but there

<sup>1</sup> Part I of this investigation was published in *Proc. Roy. Soc., Victoria*, xxxii (new series), 1920.

has been, on the whole, little advance in our knowledge of the actual relations existing between the fungus and the higher plant, although many hypotheses have been put forward.

The Gramineae as a family has been thought to be practically immune from mycorrhizal invasion. Schlicht (30) described a mycorrhiza for *Holcus lanatus* and also for *Festuca ovina*. Janse (11) examined the roots of a *Paspalum* and found them to be non-infected. Just as this paper was being prepared for the press an abstract appeared in the 'Review of Applied Mycology' of a paper by Peyronel (24), in which he describes an endotrophic mycorrhiza in a large number of wheat crops examined by him in Italy. The same fungus was also found associated with the roots of oats, barley, rye, and maize, as well as in several weeds of cultivated fields. Since the *Loliums*, in addition to the above forms, are not autotrophic in their method of nutrition, it is possible that the existence of mycorrhiza in Gramineae is a much more widespread phenomenon than has hitherto been thought to be the case. This is a fact of deep significance, as a knowledge of the exact methods of nutrition of crop and pasture grasses will undoubtedly help towards a proper understanding and practical application of the best growth conditions for these forms.

In Part I of this paper (22) the mode of growth of the fungus in the developing *Lolium* plants was likened in some respects to the behaviour of a fungal association which occurs in *Calluna vulgaris*. The latter plant possesses, as Rayner (26) has shown, a definite mycorrhizal felt on its roots; at the same time the fungus forming this union grows in the tissues of the maturing plant and enters the carpels, when they are formed, growing with them and finally infecting the seed-coats, where it remains dormant until suitable conditions ensue for germination; then, when the young root issues from the seed, the fungal hyphae present in the coat infect the new plant and thereby establish the right conditions for further development, as the symbiosis in this case is obligate. The fungal partner found in *Lolium* runs a closely parallel course in the tissues of the grass plant. The similarity of behaviour between the higher plant and the fungal symbiont in both cases seemed rather suggestive, but at this time no mycorrhiza was known to exist for *Lolium*, and Ramsbottom (25) pointed out, 'Since the examination of *Lolium* roots shows no typical endophytic fungus present . . . though it would appear at first sight that the progress of evolution had been along a line similar to the *Calluna* type leading to infection of the embryo as apart from the seed-coat and consequent continuous infection, it is more likely that in the typically non-mycorrhizal grasses such a union has been brought about by a subjection of a seed parasite'.

The establishment of the presence of a mycorrhiza must modify the above statement and bring the *Lolium* plant more into line with the *Calluna* type. No attempt has yet been made, however, to connect the

mycorrhizal fungus with that which occurs in the aerial parts of the plant, and until this has been accomplished it is not possible to state that the fungus present in the *Lolium* grains is in any way connected with the perpetuation of a mycorrhizal association such as is the case in *Calluna*. It, however, opens up an interesting line of work.

The mycorrhiza in question is a particularly favourable one for studying the cytological detail in the infected cells. As a result I have been able, at a later stage in this paper, to contribute in some measure to our knowledge of the 'exchange relations' between the two members of a mycorrhizal union, a point round which there has centred much discussion, resulting in varied and opposed hypotheses being put forward, but about which no finality has been reached.

#### METHODS.

The presence of an endophytic mycorrhiza has been noted in *Lolium temulentum*, L., *Lolium perenne*, L., *Lolium multiflorum*, Lam., and *Lolium subulatum*, Vis.; an intense cytological examination has been made only for *Lolium temulentum*. The behaviour of the mycorrhiza in the other forms, as far as a cursory examination can reveal, is closely related to that found in Darnel. The roots were fixed in Flemming's strong mixture, Carnoy's and Bouin's fixatives (17). The first-named fixative gave the best results, especially when used under reduced pressure, for the air in the roots is very difficult to displace at air-pressure. Carnoy's fixative was useful in the later part of the work. Microtome sections were employed, stained principally with Heidenhain's iron-haematoxylin counterstained with eosin, or fuchsin-iodine green. Roots cleared with carbol-alcohol and examined entire were useful in studying the distribution of the endophyte.

#### THE MYCORRHIZA OF *LOLIUM TEMULENTUM*, L.

##### A. *The distribution of the endophyte.*

Although often present in the older and larger roots, the mycorrhiza occurs most abundantly in the finer branches of the fibrous root-system, where the cytological detail can best be studied, for in the former place it has most often reached the last stage in its development. In roots of about 0.75 of a mm. in diameter the distribution of the fungus is very characteristic. The histological structure of the *Lolium* root agrees with that found in most grasses and which has been described and figured by van Tieghem (35). The epidermis, with its accompanying root-hairs, and the subjacent layer, which in transverse section is seen to be composed of rather large hexagonal cells, slightly elongated in a radial direction and closely fitted together without intercellular spaces, are always devoid of



hyphae, excepting of course those cells through which the hyphae enter the root from the exterior in a manner to be described later. The next root layer is composed of cells smaller in diameter and more rounded in outline. These cells, when the root is infected, are always filled with the mycelium of the fungus.

The cortex, bounded on the outside by these three layers, consists in the middle region of large air-spaces which have been formed by the disorganization of some of the loosely fitting rounded cells of this region. The great bulk of the cortex is here occupied by large and rather irregular spaces, but crossing at intervals are strands of cells which have remained intact and support and link up the outer layers with the inner central zone. The inner cortex is usually composed of two to three rows of rounded parenchymatous cells much smaller in diameter than those of the outer layers and displaying well-marked intercellular spaces. The endodermis is characteristically thickened and encloses an eight-arch stele. The fungus gains the inner cortex by way of the persisting strands of cells and spreads through the two or three inner layers of parenchymatous cells, but is checked in its spread by the endodermis (Pl. III, Fig. 11). The finer roots, e. g. those of approximately  $550\mu$  in diameter, although agreeing closely in structure, possess a six-arch stele with a single large median vessel. The lacunae in the middle cortex are not so well developed, and in such roots the fungus does not spread extensively in a transverse direction until the travelling strands reach the two or three layers of inner cortical cells. Although each root-system I have examined contained the fungus, infection is not universal for each individual root; some are devoid of the fungus for their entire length, while others show infected patches alternating with uninfected regions. The area which can be reached through the agency of a single penetrating strand is not very great, and if the root displays the mycorrhiza for any great distance it is due to the fact that many penetrating hyphae have gained admittance to the internal tissues.

Gallaud (9) has grouped endotrophic mycorrhizas into four series. His grouping is based on characters belonging sometimes to the endophyte and sometimes to the plants which shelter it. The first series is separated from the others very largely because the mycelium, which is at first intracellular—in the protective layers of the root—becomes both inter- and intra-cellular in the deeper layers; in the three remaining series it is entirely intracellular. The plant he studied in greatest detail which showed this form of mycorrhiza happened to be *Arum maculatum*, and he therefore called this series the 'Series of *Arum maculatum*'. The mycorrhiza of *Lolium temulentum* belongs to this series, for the hyphae, although intracellular when passing through the epidermis and subjacent layer, become intercellular and spread between, as well as through, the cavities of the cells when they gain the middle and inner cortex.

For descriptive purposes it is convenient to divide the cortex of the *Lolium* root into three parts:

- (a) *First-region*, including the epidermis and subjacent layer, which is associated with the infection of the root.
- (b) *Second region*, comprising the next two or three layers of cortical cells and associated with the spread of the fungus in all directions in the root-tissue.
- (c) *Third region*, the extent of which is naturally dependent on the size of the root (as described above), but always comprising the two innermost cortical layers (in the larger roots other cell layers are involved) and associated with the formation of sporangioles; it is also the seat of activity when the food exchange takes place between the two organisms.

B. *Mode of entry of the fungus into the root, and its behaviour in the first region.*

The hyphae which are present in the soil when they come into contact with the root can enter it in one of two ways, either way being equally common. (a) If the filament reaches a root-hair it may penetrate its wall and grow through the cavity until it reaches the corresponding epidermal cell (Pl. II, Fig. 5). Sometimes the hypha before penetration may twist round the hair in one or two close loops, but it more usually enters it directly, and, as far as I have observed, it is not accompanied by an ingrowth of the wall in the form of a sheath such as has been described for other plants by Lang (16), &c. (b) The filament may enter the root by piercing the epidermis directly; it is then usually rather inflated and misshapen at the point where the wall is actually crossed (Pl. III, Fig. 12). Characteristic passage cells are not developed in these roots; any cell of the epidermis may function as the first cell to house the advancing endophyte. Sometimes the hypha creeps along the surface of the root, putting out several short processes which lie in close contact with the outer walls of the epidermal cells, one of which eventually succeeds in entering the cell, when it becomes the infecting strand.

The hyphae exterior to the root are septate, with rather thick brown walls, but whenever they succeed in gaining entrance into the root itself these characters alter, septa become rare or absent, and the walls become thin and colourless; at the same time, however, the dimensions of the hyphae increase considerably. Having reached the interior of the root, the filament twists in a loose spiral fashion in the lumen of the epidermal cell and may, at the same time, give off short abortive horizontal branches, which do not reach usually even the neighbouring epidermal cells. The direction of growth in this first region is at right angles to the root surface and the next layer of root-cells is infected before there is any considerable horizontal

extension of the fungus mycelium in the root. The nuclei of infected epidermal cells are markedly increased in size compared with those of uninfected cells, and they seem to stain very evenly with iron-haematoxylin (Pl. III, Figs. 13 and 14). Even when the hyphae in these outer layers are empty and shrivelled, at a time when the more internal root-cells show an advanced stage in infection, the nuclei retain this enlarged condition (Pl. III, Fig. 13); they always lie closely against the loops of hyphae and are generally spherical in outline.

*C. The histology of the mycorrhizal association for the second and third region.*

*Second Region.*

The hyphae in the epidermis and outermost cortical layers are intracellular in character, but with the horizontal spreading of the mycelium in the second root layer branching takes place, and some of these branches penetrate the cell-walls and come to lie in the intercellular spaces, spreading in all directions; in this way it is easy for the hyphae to carry infection through a comparatively large area of the root-tissue. At the same time many of the branches remain intercellular and do not seem to be hindered in their course by the cell-walls, which they penetrate with ease, the hypha usually becoming constricted when passing through the dividing walls (Pl. III, Fig. 15). The function of the hyphae in the second region is to extend the infection horizontally and vertically in the root. In the smaller roots the two or three inner rows of cortical cells are soon reached, and it is there that the fundamental part of the mycorrhiza is encountered. In the larger roots the middle cortex, which consists of large air lacunae crossed by strands of parenchymatous cells, houses the endophyte in both the inter- and intra-cellular position. Some of the cells in this region, as well as those of the two or three inner layers, take part in the digestive processes which characterize the later stages of this association.

The contents of the hyphae in both the second and third region vary according to the age of the mycorrhiza. In young associations the hyphae are large and practically filled with oil or fat, which blackens densely with the osmic acid of Flemming's solution, so that these hyphae form a very striking feature in a tangential longitudinal section of a root in this region at this period (Pl. I, Fig. 1). Occasionally free drops of fat, which are also blackened by the osmic acid, are encountered in the outer cortical cells. This fat has probably escaped from the hyphae which run through the cells, either by exudation through the thin walls of the filaments or by the bursting of the hyphae owing to the pressure developed by the tremendous accumulation of reserve fat (Pl. III, Fig. 16). At a later stage in the mycorrhizal formation these hyphae are found to be practically devoid of fat and

appear empty, more or less shrivelled, and contain only remnants of their protoplasm.

Vesicles are found associated with the hyphae in this second region.

*Vesicles.* These structures are plentifully developed; more especially do they occur in an intercellular position, although some have been noticed within the interior of the *Lolium* cells (Pl. III, Fig. 17). They are oval in shape and average about  $65 \times 45 \mu$ , and are most often borne terminally on a hypha, but occasionally they are found in an intercalary position (Pl. II, Fig. 3); in such cases the hypha enlarges suddenly to form a vesicle, but later its wall softens at a point which buds out and continues to grow in the form of a normal filament. When intercellular in position the adjacent cells are often distorted, owing to the pressure of the vesicle against their walls (Pl. III, Fig. 17). When first formed these swellings are highly protoplasmic and contain abundant supplies of reserve food-material in the form of fat or oil, and in unbleached preparations made from material fixed in Flemming's solution it is often difficult to make out their structure, owing to the intense blackening of the fat with osmic acid. The mature vesicles usually have thickened walls. At a later stage in the association the majority appear collapsed and contain many vacuoles in their cytoplasm; the fat has been removed from them and transferred to the cells, where digestion is proceeding, and here it is given over to the higher plant (Pl. II, Fig. 2).

In a portion of a root, which was sectioned in the usual way, several vesicles were found which had been crushed and burst open during the manipulation of the tissue, and their contents had partly escaped into the cell-cavity. The contents in this case were different from those usually observed, consisting of a number of small spherical bodies, each of which appeared to be nucleated, the whole resembling a sporangium with contained spores (Pl. II, Fig. 4). Bernatsky (5), working with *Psilotum*, recognizes the vesicles of this plant as sporangia which have been arrested in their development, and he identifies them with the sporangia he has obtained on a *Hyphomyces* from *Psilotum*; others compare them with the oogonia of *Pythium*. However, the reasons given for associating the endophyte with any definite fungal species by all these authors are very debatable.

The majority who have studied mycorrhiza have looked upon vesicles, which are so generally met with in endophytic mycorrhizas, if one excludes the Orchidaceae, as reproductive organs or kinds of cysts where nutritive material accumulates and which persist in the soil after the destruction of the root, and by later germination give rise to new mycelia capable of infecting fresh roots and thus contribute to the propagation of the endophyte. Any attempt, however, to induce the vesicle to germinate and produce a mycelium under artificial conditions has failed (Bernard (4)).

From an examination of the roots of *Lolium* the function of the vesicle appears to be primarily, from the point of view of the fungus, an attempt

towards spore formation. This is borne out by the increase in the number of nuclei and the dense protoplasmic contents, coupled with abundance of food material. In comparatively few instances, however, is this end realized. The specimen figured on Pl. II, Fig. 4, probably represents a vesicle which has succeeded in attaining this objective, and the spores formed in its interior could germinate in the soil after the rotting of the root and so propagate the endophyte. In the majority of cases, however, the food material which is present in such abundance in the hyphae of the second region, including the young vesicles, during the first stages of a mycorrhizal invasion, is, as will be seen later, rapidly carried to the cells of the third region, and here is given over to the host-cells, the fat in the young vesicles being removed along with that of the vegetative hyphae. The vesicle is consequently deprived of food material and cannot therefore proceed with its development, so at this stage a great number appear collapsed and empty in the root-tissue, in just the same way as the penetrating and travelling hyphae do in the outer layers of the root. So, from the point of view of the higher plant, the vesicle in most cases is simply a temporary reserve organ functioning as such during the early stages of the fungal invasion.

### *Third Region.*

As has been indicated above the extent of this region is variable, depending on the size of the root examined; the occurrence of sporangioles delimits it from the second region. In roots of 0.75 mm. diam. and upwards, in which the lacunae of the middle cortex are well developed, arbuscules with their accompanying sporangioles may be formed in the last of the three outermost cortical layers, as well as in some of the cells forming the connecting strands of the middle cortex, but they reach their maximum development in the two or three inner cortical layers which abut directly on the stele of the root (Pl. III, Fig. 11). In roots of smaller diameter the middle cortex is not so well developed, and the formation of sporangioles is restricted to the inner cortical layers.

*Arbuscules and sporangioles.* These together constitute the most important organs of the endophyte. Although Janse (11) had at an earlier date described and figured sporangioles in many plants containing mycorrhiza, Gallaud (9) was the first to draw attention to the regular dichotomous type of branching uniformly indulged in by the intracellular branches of the hyphal filaments in the deeper layers of the roots of such plants (excepting those of the Orchid family) and on which the sporangioles are borne. He gave the name of arbuscules to these structures, and, although all arose in essentially the same manner, he found that in some plants they were comparatively simple in form, while in others they were complex and formed a striking network of fine branches in the host-cell. The fungus in the *Lolium* roots is characterized by possessing simple arbuscules. In Darnel

they may arise either from a short intracellular branch of an intercellular filament (Pl. III, Fig. 18), or from lateral branches of a main intracellular hypha (Pl. III, Fig. 22). In either case the branch divides, at first perhaps irregularly, spreading in this way through the cell cavity, but finally there is produced a few dichotomous branches forming small, rather inconspicuous terminal tufts of fine hyphal threads. The arbuscules of the *Lolium* roots are very ephemeral, quickly being resolved into sporangioles, and they are in no way a prominent part of the association. The sporangioles,<sup>1</sup> on the other hand, are easily demonstrated (Pl. III, Figs. 18, 19, 20, and 21); they appear to remain intact for a short period after their formation, and they are coloured very strongly with fuchsin after staining with fuchsin-iodine green mixture, and a reddish purple after iron-haematoxylin. Their appearance varies with their age, due to the fact that they pass through a series of changes, which end in their disruption and the consequent escape of their contents into the cell-cavity. Sections made from material gathered in early September, when the grass was just forming the inflorescence, show the sporangioles in the young condition (Pl. III, Figs. 18 and 19). In this state, they appear as the irregular swollen ends of the fine arbuscular branches (Pl. II, Fig. 6). Owing to their position, they occur in clusters, and there may be several clusters in the same cell. The hyphae bearing the arbuscules and sporangioles are, during the early development of the latter, filled with oil, which blackens intensely with osmic acid, but as the sporangioles increase in size and reach their mature condition, the oil is transferred to these organs; the supporting hyphae appear practically empty, only containing a little protoplasm and some nuclei (Pl. II, Fig. 6).

The sporangioles vary widely in shape, but they usually agree in possessing an uneven, more or less papillated surface, due to the oil globules in their interior, which cause the wall of the sporangiole to project in a manner corresponding to the shape and position of the globules (Pl. III, Fig. 23). When the oil is transferred to the sporangiole, it appears to become altered in some way, for it no longer gives a black coloration after treatment with the osmic acid of Flemming's solution: in fact, if sections made from material fixed in Flemming are examined without staining, the sporangioles appear as yellow or light brown refractive bodies in the cyto-

<sup>1</sup> Janse (11) and Gallaud (9) use the term 'sporangiole' in rather a different sense. Janse, who discovered and gave them their name, recognized them to be definite bodies formed by the mycorrhiza, which, when they later undergo change by digestion, or some other means, lose their identity, and become transformed into a structureless mass in the cell-cavity. Gallaud recognizes at one point in his paper two kinds of sporangioles: (a) those with a clear and well-defined mammillated outline, and (b) others with a cloudy or floccose appearance formed by fine granulations, evidently the result of the digestion of (a). Judging from this differentiation he did not restrict the term to the undigested hyphal organs as meant by Janse, as is borne out at a later stage in his paper, for he says the sporangioles are only the residue of a more or less advanced digestion. The significance of the 'sporangiole' as a fundamental stage in the association is lost if this view be admitted. In this paper the term is used in the sense conferred upon it by Janse and affirmed by the writer.

plasm, showing a negative result with the osmic acid of this solution. If such sections are stained with iron-haematoxylin, it is often possible to see the fat present inside the sporangiole, for it stains black with the haematoxylin, while the wall and cytoplasm of the sporangiole give a reddish-purple colour (Pl. III, Fig. 19; also Pl. II, Fig. 6).

The cell nucleus, during these changes, is always closely applied to the fungal threads, more particularly to the sporangioles, and it is often difficult to focus, for it is usually overlain by these organs (Pl. II, Fig. 6, and Pl. III, Fig. 22). It is much enlarged, often lobed and irregular in shape, and always shows a very distinct chromatin network and nucleolus (Bernard (3)); at the same time the cytoplasm of the host-cell is intimately connected with the sporangiole masses. In sections of material gathered at this time, the *cell inclusions then consisted of the hyphae with their sporangioles, cell nucleus, and cell cytoplasm* (Pl. III, Fig. 12; also Pl. I, Fig. 1, and Pl. II, Fig. 6).

If sections made from roots gathered in late October and early November, when the Darnel grass is forming grain, be examined, many differences may be noted. The sporangioles, when they have reached maturity and contain all or most of the oil of the accompanying hyphae, disrupt in some way, possibly by the action of the host-cell on their wall, and their contents escape into the cell-cavity. (Pl. III, Fig. 20 shows a sporangiole which has been artificially ruptured.) The cell then contains the globules free in its lumen (Pl. III, Fig. 23). They are homogeneous in character and rounded in outline; the size of these oil-bodies varies (from 1–20  $\mu$  in diam.) even when first set free from the sporangiole, but at the moment of liberation they are usually small (Pl. II, Fig. 8). Sometimes they remain at their initial size, and then the cell contains a great number of them, the number really depending on the number and size of the original sporangioles. More often, however, they run together after liberation, coalesce, and form a smaller number of large globules, sometimes reaching a size of 25  $\mu$  (Pl. III, Fig. 23; also Pl. II, Fig. 7). The sporangioles of any particular cell are not necessarily all at the same stage, and one often encounters a cell containing intact sporangioles and sporangioles collapsed and sunken, from which the fat has been ejected and which lies free in the cell-cavity (Pl. III, Fig. 23; also Pl. II, Fig. 7). These free globules also give a negative result with the osmic acid of Flemming's solution; the smaller droplets remain quite uncoloured, but the larger of them may be faintly brown after such fixation.

In sections stained with iron-haematoxylin the globules stain deeply and appear black in colour. In fuchsin-iodine green preparations the young intact sporangioles stain pink, but the free globules in the cell are coloured green, and they form a very striking and conspicuous feature in such sections, the depth of colour of course depending on the size of the droplet.

In sections of material gathered later in the season the *cell inclusions consist of shrivelled and empty hyphae, disorganized sporangioles, cell nucleus, cell cytoplasm, and a large number of variously sized oil globules* (Pl. II, Figs. 7, and 8; cf. Pl. III, Fig. 22).

The free fat content of the cells reaches a maximum when all the sporangioles of that cell have been ruptured. The burst sporangioles appear as rather indefinite granular masses in the cell cytoplasm. The fat is then gradually removed from the cells of the third region: it is probably transported in some form to the region of active growth, i.e. to the developing grain. While the fat is disappearing, the fungal mechanism in the cell, which consists of the collapsed hyphae and the remains of the sporangioles, undergoes a further change: these remnants appear to be digested by the host-cell. The smaller fungal branches and empty sporangioles lose their identity and are resolved into a dense granular mass, which often encloses some of the fat globules, not as yet removed from the host-cell; the mass stains deeply with the haematoxylin and fuchsin stains (Pl. II, Fig. 9). It is intimately associated with the cell protoplasm and the cell nucleus: the latter retains its size and staining capacity throughout the above changes, and is most often situated in the centre of this disorganizing body. Any fat droplets enclosed at the beginning of this disorganization are removed before it is completed. The cytoplasm at this time is granular, and forms a dense reticulation in the cell.

In sections made from material fixed in November, free fat globules are no longer visible; the contents of the cells of the third region, at this time, agree throughout the infected portions of the root, and consist of a dense reticulated material with the nucleus still large and forming a prominent part of the cell contents. This reticulated body finally becomes transformed into a structureless slimy-looking mass, which fills up the cell-cavity. Sometimes this structureless residue is crossed by an empty hyphal filament, which retains its shape and size throughout the changes which have taken place in the cell (Pl. III, Fig. 24). The hyphae which do this are always of large dimensions, and in the earlier stages of the association formed part of the main conducting tract to the inner cells. They possibly have been able to resist digestion because their walls may have become altered in order to afford support to the main hyphal filaments. The cell mass stains lightly and evenly with haematoxylin; the nucleus is embedded in the centre of it, and is even at this stage usually larger than those of normal uninfected cells; the cells appear to remain in this condition until the death of the root.

#### D. *The fat globules of the third region.*

In order to make the description of the histology of the second and third regions more continuous, I alluded to the globules, found in the interior



of these cells at a certain stage in the mycorrhizal union, as fat globules (Pl. III, Fig. 23; also Pl. II, Figs. 7 and 8), without entering into a discussion of the reasons which have led me to regard these as such. However, in this section I propose to deal more fully with them, and to set down my reasons for reaching this conclusion. The globules are present in such numbers, and form such an outstanding feature of the cell inclusions during the 'exchange stage' that they could not readily be overlooked, and, moreover, it is obvious that they have passed into the cell from the enclosed fungal mycelium via the sporangioles. The first sections which were examined had been made from material fixed in Flemming, and as the globules remained uncoloured, even though they had been subjected to osmic acid in the fixative, in contrast to the fat in the penetrating and travelling hyphae which blackened strongly, it was thought they were not fatty in nature, and the possibility of their carbohydrate character was first investigated.

Sections, made from fresh roots and from roots which had been previously fixed, were stained with potassium iodide-iodine, but the globules remained unaltered. From material which had been fixed in Carnoy's solution a triplicate set of slides was prepared, and they were used to test for the presence of glycogen. After fixation and subsequent washing in absolute alcohol, the material was embedded in paraffin and sectioned in the usual manner. These sections were applied to the slide with a mixture of 50 per cent. alcohol and a small amount of glycerine and albumen, using the alcohol as one would water. One slide was then subjected to the *iodine technique*, as set out in Lee (17). The second, after standing in 1 per cent. celloidin overnight, was transferred, after preliminary treatment, to Ehrlich's haematoxylin, differentiated in acid alcohol, and then stained with Best's carmine stain (see Lee, p. 295). The third slide was spat upon and set aside, in order that the saliva might have a chance to act, and the slide was then stained with Best's carmine. The negative results obtained excluded the probability of *glycogen*.

Similarly, protein tests gave negative results.

In view of the above facts, and also on account of their globular shape and homogeneous appearance, as well as the fact that the hyphae when young are packed with fat, which disappears from them when the sporangioles are mature and ready to yield up these bodies to the host-cell, the idea of their fatty nature seemed so strongly supported that further tests to throw light on this point were then resorted to. Lee (17) includes a chapter written by Dr. W. Cramer on fatty substances, in which he enumerates and tabulates the histochemical fat reactions. Dealing with the osmic acid methods, he states that the *true fats and lipoids* are all blackened by osmic acid, due of course to the fact that these substances, since they possess a double linkage in their molecule, are more or less easily oxidized. He states that 'the various groups of substances differ in the readiness with

which they are oxidized, and consequently in the rapidity with which they are blackened by osmic acid and the depth of blackening produced'. True fats are readily blackened, but other fatty substances form a series in which there is a gradual reduction in their response to the oxidizing action of osmic acid. This difference in the reducing power of these fatty substances can be accentuated by using *osmic acid together with bichromate* solutions, the reason being that the bichromate itself acts on the various double linkages in the molecules, and so prevents the osmic acid from being reduced, except by true fats and a few other fatty substances which have a strong reducing power. Since Flemming's fixative is a bichromate-osmic mixture, the non-blackening of the globules in question after such fixation will not therefore exclude them from the fat series, but only shows they are not true fats. Further tests are required if they are to be entirely removed from this category. The following tests were then made:

(a) In sections made from material fixed in Flemming and stained with haematoxylin, it was noted, as has already been pointed out, that the globules stained an intense blue-black. This is the reaction given by fatty substances after such treatment, for the chromium compound formed by the fat with the bichromate has the property of forming a dark blue lake with haematoxylin, and is used to demonstrate the presence of fatty substances in sections, for it gives good histological detail.

(b) Portions of fresh roots of small diameter were then taken and stained with Sudan III. This reagent was allowed to act for a considerable time, and then the specimens were mounted in glycerine. Microscopic examination showed that the globules, which could be readily seen in the inner cells, appeared red in colour. True fats, cholesterin esters, and cholesterin-fatty acid mixtures are characterized by this reaction.

(c) Portions of fresh roots of similar size were then subjected to osmic acid alone, and the globules were seen to blacken, though rather slowly. All fatty substances agree in this reaction.

(d) In sections of roots stained with fuchsin-iodine green the globules form a striking part of the cell contents, for they stain vividly with the green stain. In order to see if this staining reaction was an indication of their chemical character, sections of castor-oil endosperm were stained with the same mixture, and it was noted that the oil immediately took up the green dye, and the globules in the endosperm stained in a similar way to the globules in the Darnel root. I do not know if this stain has ever been used as a test for fat, but, as far as my experience goes, it seems to be a ready indicator of the presence of such in a plant-cell.

(e) Finally, roots of Darnel were fixed in Carnoy's solution. This solution contains chloroform, which is a fat solvent. Sections were made in the usual way and no sign of the globules could be seen; moreover, the immature sporangioles were shrunken and useless for histological work,

probably because the fat had been removed from them by the action of the chloroform.

The tests enumerated above show that the globules which are found in the root-cells at a certain stage in the mycorrhizal association are *fat globules*. Since the fat in the travelling hyphal filaments stains black with osmic acid after bichromate, it is therefore a true fat, and some change must take place in its constitution before it is liberated into the interior of the cell in the form of globules, for in this condition it no longer responds to the tests which are specific for true fats.

As the globules described and figured by Kusano in his paper on *Gastrodia* agree in staining reactions, shape, and position with the fat globules found in the *Lolium* cells, it is interesting to consider briefly this paper. *Gastrodia elata*, Bl., seems to differ, as regards the organization of its mycorrhiza, from the majority of Orchids. Gallaud (9) grouped these latter plants together to form his fourth series of mycorrhizal types. This group is characterized by the formation of 'clumps', formed by hyphae of uniform diameter in the host-cells, each clump being connected by a hyphal branch to a clump in the neighbouring cell; some of the clumps later undergo transformation and form an indistinct mass or degenerated body in the cell lumen. However, both Janse and Gallaud insist on the ultimate resemblance between these bodies and the sporangioles of other endophytes. Kusano (15), in his paper on '*Gastrodia elata* and its Symbiotic Association with *Armillaria mellea*', divides the cortical region of the tuber of this plant into three parts, according to the structure of the cells and the nature of the hyphae contained in them. Judging from Pl. 3, Fig. 17 of his paper, the hyphae in the cells of the first region 'clump' in a manner similar to that found in other Orchids, but they do not undergo digestion. *Gastrodia elata* differs from the rest of the Orchidaceae, in that the centre of the metabolic activity is situated in the third or inner region of the mycorrhizal zone, where the characteristic clumping is no longer found. Kusano states that 'the infection of the hyphae (in the cells of the third region) induces a dense granular appearance of the cytoplasm formerly reticulated, and at the same time a further increase of its amount. The hyphae are then embedded in the cytoplasm and make no noteworthy development.' Although he recognized this third or inner region to be the seat of metabolic activity, and found, as a result of it, secondary products in the cytoplasm of these cells, he apparently did not find, although clumping was absent, any special fungal mechanism (such as sporangioles) through which these products were liberated into the host-cell. In this connexion, it is interesting to refer to the figures accompanying Kusano's paper illustrating the cytology of the cells of this digestive zone, more particularly Pl. 3, Fig. 24, and Pl. 4, Figs. 43, 49, and 50, which seem to me to suggest the presence of organs analogous to sporangioles, although not recognized as such by Kusano. In

the present state of our knowledge, *Gastrodia elata*, Bl., is exceptional among Orchids as regards its mycorrhiza, and if these bodies are really sporangioles, it would form an interesting link between Orchids and the endophytes of other plants. Moreover, it should be noticed that the hyphae of the cells of the second region in *Gastrodia* finally collapse or shrivel, and their contents are reduced to a densely staining string; these filaments are in communication with the hyphae of the third region, and there seems to me no reason to doubt the fact that their contents are transferred to these inner cells and later appear as the secondary products in the cytoplasm of these cells, for exactly this change is noted in the travelling hyphae of the Darnel root. The secondary products, which appear in the interior of the active metabolic cells, have been named by Kusano homogeneous globules, vesicles, and small bodies. It is with the first of these that I wish to deal. He describes the globules as hyaline spherical masses, light yellowish brown in colour, varying in number and size in the individual cells, staining green with fuchsin-iodine green, and black with haematoxylin. This description and their exclusive occurrence in the active digestive cells, as well as their origin—for Kusano is of the opinion that 'they are produced undoubtedly by the secretion process going on in the hyphal filament'—lead me to the conclusion that the homogeneous globules of Kusano are identical with the fat globules of the *Lolium* mycorrhiza; cf. also Pl. 3, Figs. 19 and 22, and Pl. 4, Figs. 27, 29, 45, 46, 47, and 49 of Kusano's paper. Kusano states that the homogeneous globules were not blackened with osmic acid, and he believed them to be protein in character, although he does not support this conclusion with any evidence whatever.

It is of interest to note at this point that a recent paper by McLuckie (23) deals with the mycorrhiza of *Gastrodia sesamoides*, R. Br., the Australian member of this genus. The results set out in this paper differ widely from those obtained by Kusano for *Gastrodia elata*, Bl. McLuckie did not find any 'suggestion of disorganization or digestion of the fungus by the cell contents'.

The biological significance of the occurrence of fat globules in the mycorrhizal cells during the metabolic exchanges will be dealt with in the next section.

## CONCLUSION.

Owing to the widespread occurrence of mycophytic plants and to the generally accepted idea that the fungi are of importance in the nutrition of these forms, much discussion has centred in the relations existing between the two components of an endophytic mycorrhizal association. Although perhaps all such associations are not identical in character, it is more than probable that the majority of them agree in essential respects, and

that the 'exchanges' encountered during the digestive phase of such an association can all be grouped into one big category, the differences met with being of degree rather than of kind. Many hypotheses, to explain these relations, have been put forward and supported by various workers on this subject. Of the more recent papers, West (37), writing on the mycorrhizas of the Marattiaceae, includes an epitomized account of some of the suggested functions performed by the union between the fungus and the higher plant. Rayner (27) and Ramsbottom (25), in his paper on 'Orchid Mycorrhiza', also give an historical account from this point of view. The controversy has been waging for so long, and so many conflicting hypotheses have been put forward, that it is impossible to deal with each in detail.

It may be said to have commenced with Frank (8) and his classic works on mycorrhiza (1885); he is responsible for the first clearly formulated theory of symbiosis between the fungus and the root, although this idea had arisen as early as 1862. His idea was that in ectotrophic mycorrhizas the mycelial mantle substituted itself for root-hairs and replaced their function; the fungus absorbed and then yielded to the plant mineral salts and organic nitrogenous food obtained from the humus; in return the host plant gave hydrocarbon material, which it manufactured, to the fungus. Frank at first claimed a similar role for endotrophic mycorrhizas, i.e. the plant gained nitrogenous material from the humus through the intermediation of the fungus, but later he pointed out the rarity of the communications of the endophyte with the exterior, and he modified his theory on this account and stated that the host plant procured *nitrogenous* food for itself by digesting the fungus which filled up the more internal cells. Frank's experiments and conclusions were practically universally accepted at this time, and they, coupled with the fact that the somewhat analogous root tubercles of the Leguminosae have been indubitably proved to be related to nitrogen assimilation, seem to have exerted a profound influence on much later work with root fungi, and the establishment of a connexion between nitrogenous metabolism and endophytic mycorrhizas is sought after at the present time.

Janse (11) drew a similar conclusion after an extended examination on many mycophytic plants. Stahl (33) placed a new interpretation on the role of symbiotic fungi; he endeavoured to establish a relation between low transpiring powers and the presence of mycorrhizas in the roots, as opposed to entirely autophytic plants, with a relatively high transpiring capacity, and from the results he obtained he considered the fungus in the root gave to the higher plant the products of assimilation of mineral salts, which it procured from the humus soil in which it flourished. Papers by Magnus (18) and Shibata (32), of a cytological character, thoroughly established the occurrence of digestion of the fungus in the host-cells, and the

latter author considered that the actual function of the union was arrived at by combining Frank and Stahl's hypotheses.

Gallaud (9) introduces a new and opposed point of view. He concludes that the fungus in the root is so isolated from the exterior, on account of the few connecting hyphae present, that it must lead an independent existence in the root-tissues and must therefore derive all its food from the host plant, being what he terms an 'internal saprophyte'. Bernard (3), departing from the ideas of all previous workers, looks upon the fungus as a parasite which is subsequently checked in its spread in the root-tissue by the action of the root-cells, which confer upon the infected plant a kind of immunity. He compares the process which takes place to phagocytosis, and in this connexion places great weight on the behaviour of the nucleus in the occupied root-cell. Kusano (15) holds that the fundamental exchange is a nitrogenous one from the fungus to the higher plant. McDougall (21) controverts Stahl's hypothesis, and states that the endophyte gets all or a large percentage of its food from the root, and, since this is so, it must be considered as an internal parasite, but he modifies this by adding that if the 'endophyte occupies only a small percentage of the cortical cells, the root may receive sufficient benefit from the digestion of fungus hyphae to justify applying the term symbiosis to the association'.

This brief *résumé* shows that up to 1914 no clear proof of the nature of the intimate relations existing between the two members of a mycorrhizal union had been brought forward, and consequently the varied and opposed views held on the subject have led to many further investigations.

Weevers (36), working from a chemical point of view, on the presence of ammonia and ammonium salts in plants, found that, although they occurred in abundance in the tubercles of the Leguminosae, they were in very small quantities or absent in mycophytic plants, and he concluded, therefore, that if the fungus roots really assimilate nitrogen it must be brought about in a manner different from that in the Leguminosae. Weevers is of the opinion that mycotrophic plants are, with the help of their fungus partner, able to utilize fully the organic compounds of the soil. Melin (19) isolated the mycorrhizal fungi from *Pinus* and *Picea*, and he found that no fixation of nitrogen took place in pure cultures of these fungi. It may also be noted here incidentally that Burgeff (6) was unable to show any nitrogen fixation in Orchids.

Summarizing these results, one may say that although many workers have suggested on theoretical grounds the function of nitrogen fixation for endophytic fungi, they have done so without any real evidence that such is the case; indeed, the bulk of the evidence brought forward up to 1916 was in opposition to this idea.

However, Rayner (28) in 1922 published a paper on 'Nitrogen Fixation in the Ericaceae', and in this holds that 'there is cumulative

evidence that the endophyte of the Ericaceae can utilize atmospheric nitrogen in greater or less degree'. This statement is based on experimental evidence derived from three sources, viz.:

(1) From Ternetz (34), who isolated eight pycnidia-bearing fungi from the roots of members of the Ericaceae; these were referred to the genus *Phoma* and were found to be capable of fixing atmospheric nitrogen in varying degrees. Since the identity of these forms with the endophytes of the roots concerned was not definitely established by the formation of a mycorrhiza after inoculation from pure culture into the roots of seedlings free from fungal infection, this work was not conclusive.

(2) From Rayner (26) herself, who isolated a pycnidia-forming fungus from *Calluna* and was able to prove that the form obtained was identical with the endophyte of this plant by re-inoculation into seedlings grown in pure culture and raised from sterilized seeds. The characters of the endophyte isolated from *Calluna* agreed with those obtained by Ternetz, and it was presumed that the forms used by the latter worker for demonstrating nitrogen fixation were actually the endophytes of the plants concerned. Rayner also experimented with pure culture seedlings inoculated at planting with the endophyte and grown under sterile conditions in media lacking combined nitrogen, and found that 'the seedlings deprived of combined nitrogen were green and healthy and grew at the same rate as the controls'. Rayner herself points out that the acceptance of this result as conclusive evidence for fixation of nitrogen by this endophyte might be objected to on the ground that the seedlings of *Calluna* could grow for several months on the seed reserves, and that this could account for the vigour and longevity shown by such seedlings as well as by seedlings supplied with distilled water only. Against this objection she uses the fact that 'seedlings germinated on moist filter-paper from sterilized seeds not only form no roots, but make practically no shoot growth and quickly show symptoms of starvation, such as yellowing and discoloration of the leaves, and that these symptoms are relieved by inoculation from a pure culture of the endophyte'. This fact does not appear to me to affect the objection raised, for the symptoms shown by *uninoculated* seedlings from sterilized seeds when grown on moist paper are due to the absence of the endophyte, the absence of which might be disturbing the metabolism of the plant in many possible directions, and is not conclusive proof that absence of nitrogen is bringing about the results.

(3) From Duggar and Davis (7), who, working on the nitrogen-fixation of fungi, took extreme care to avoid experimental errors. They isolated forms of *Penicillium*, *Aspergillus*, *Macrosporium*, *Glomerella*, and *Phoma* from different soils, and found that the first four could utilize free nitrogen to a very slight extent; the amount is so small that Duggar could not accept it as evidence of ability to fix nitrogen; however, the values got for the latter

form in a culture media with a known amount of combined nitrogen supplied were higher and comparable to the values obtained by Ternetz for the species of *Phoma*, and seem to be evidence that the power of nitrogen fixation in varying degrees exists in the genus *Phoma*.

These results are thought to provide a basis for the explanation of the relations existing between the endophyte and the higher plant, at all events as far as the Ericaceae are concerned. Such is the position at the present time.

The results set out in this paper suggest another interpretation of the physiological relationship existing between the fungus and the Darnel grass, and it seems that this interpretation is not inconsistent with the results obtained by workers who have experimented along distinct but closely allied lines. It has been shown that the development of mycorrhiza in the grass root culminates in the formation of sporangioles in the more internal cells. At the same time the fat or oil in the hyphae responsible for the extension of the mycorrhiza in both a radial and tangential direction in the root is concentrated in these organs, and, at a certain stage in the association this is given up to the host-cell through the disintegration of the sporangiole wall and comes to lie in the cell-cavity. This is utilized by the higher symbiont, as is evidenced by its removal from the cell until finally the latter is occupied by the characteristic inert residual mass without any sign of fat or oil which previously was a dominant feature of the cell inclusions. Accompanying these changes, the rest of the fungal mycelium in the root becomes collapsed and empty, quite obviously no longer forming an active part of that structure and excluding the possibility that the transference is from the root to the fungus, for the collapse synchronizes with the appearance of fat in the cells.

From these observations it appears that the exchange in this case is carbonaceous rather than nitrogenous in character, the fat or oil being given over to the green plant for use in its nutrition.

One of the chief objections raised by the earlier workers on mycorrhizas against the view that the higher symbiont gained food from the soil via the fungus was the reported poverty of communicating channels through which this exchange might be maintained, but as far as my observations go this can no longer be held as an objection, for the penetrating strands, though not as numerous as root-hairs, in Darnel are nevertheless plentiful and would be an efficient means of communication between the soil and the interior of the root. So, from a cytological examination of the root-tissue, evidence is gained which supports the hypothesis *that the fungus is used by the root as a source of carbonaceous nutriment, and the fungus apparently derives little or no benefit from the association.* The idea of mutual advantage is not borne out by an examination of the mycorrhiza of Darnel: rather does it seem that the green plant is parasitic on the lowly member, being the aggressive partner of the union.



This conclusion is given support by a consideration of the results obtained by Knudson (14), working on the germination of Orchid seeds—a notoriously difficult proposition. Bernard (2) in 1909 had produced evidence tending to show that germination of these seeds and the growth of seedlings are dependent upon infection by certain strains of fungi which are found living in Orchid roots. Burgeff (6) reached practically the same conclusions, maintaining that germination was only accomplished when the embryo became infected with the right fungal strain. However, Bernard later succeeded in germinating seeds of *Cattleya* and *Laelia* without the intervention of the fungus, by sowing them on a more concentrated solution of salep.<sup>1</sup> The germination by this means was good and the seedlings obtained were normal in every way. Knudson (13), having previously demonstrated that various sugars have a very favourable influence on the growth of plants, and in view of the results obtained by Bernard, decided to test the effect of the use of certain sugars in sterilized culture media on the germination of Orchid seeds, and gained remarkable success, and he was forced to the conclusion '*that the germination of seeds of Cattleya and Laelia is possible without the fungus provided some soluble organic substances are present, particularly sugar*'.

Knudson is of the opinion that the influence of the fungus on the germination of seeds is probably *external* in character, and in view of the above statement says, 'It is conceivable that germination is induced, not by any action of the fungus within the embryo, but by products produced externally on digestion or secreted by the fungus'; but later he states that certain facts gleaned from Burgeff's experiments make it difficult to explain the action as purely external in nature.

Miss I. C. Cookson, of Melbourne, has repeated Knudson's experiments, using seeds of Australian terrestrial Orchids, including representatives of the genera *Pterostylis*, *Caladenia*, *Microtis*, &c., with similar results, obtaining in most cases almost 100 per cent. germination. Her work has not been published as yet, but I have had the privilege of examining the culture flasks at various stages of experimentation.

The success encountered in the non-symbiotic germination of Orchid seeds is due to the presence of *soluble carbonaceous* food in the culture media, which compensates for the absence of the fungus, if the function of the latter is, as is borne out by the cytology of the *Lolium* mycorrhiza, to *supply carbonaceous material to the host plant*. The fungus, of course, is independent of this supply of *soluble* sugars under natural conditions, owing to its enzymic powers, but it can absorb the products of such activity and hand them on to the higher plant.

<sup>1</sup> *Salep* is a powder obtained by pulverizing the dried tubers of various species of Orchidaceae, and contains chiefly mucilage 48 per cent., starch 27 per cent., and proteins 5 per cent.; it probably contains also some sugar, as well as soluble mineral matter.

Finally, I would point out that experiments carried out by Knudson to show the effect of increased concentrations of sugar in the culture flasks containing the Orchid seeds led to the discovery that concentration of glucose beyond 0.80 per cent. is without significant effect. When a microscopic examination was made of the Orchid embryos from cultures containing up to 2 per cent. concentration of glucose, however, an interesting fact correlated with this result was established. Starch was found to be present only in those embryos growing on culture media with a concentration of 0.80 per cent. and higher. As Knudson points out, this fact is evidence that the absorption of glucose, at a concentration of 0.80 per cent., is in excess of its utilization, and then it appears in storage form in the embryo. Bernard (1) has suggested a close relationship between *tuberization* and the presence of endophytic fungi in plants. It is a striking fact that a great number of mycosymbiotic plants tend towards a tuberous habit, or to develop bulbs and protocorms. Bernard was so convinced of the existence of this relationship that he sought to, and did, establish the presence of an endotrophic mycorrhiza in the members of the genus *Solanum* growing wild in France, in order that he might thus explain the origin of the tuber of the cultivated potato.<sup>1</sup> The result obtained by Knudson, when growing seeds of Orchids on media containing varying quantities of sugar, seems significant in this respect, for if an excess of carbonaceous material is available it is stored up in the tissues of the plant, and it is conceivable that the tuberous habit, which is so extraordinarily widespread in plants with endophytic mycorrhizas, may have been brought about by the large amount of such material which can be obtained by the plant through the intermediation of the associated fungus.

A micro-chemical examination of the endotrophic mycorrhiza of *Lolium temulentum* (L.) reveals, then, that the physiological function of the union is associated with the supply of material rich in carbon to the higher plant. In this particular case, and possibly also in *Gastrodia elata*, the material given up is in the form of fat or oil. Moreover, this idea throws some light on the results obtained by other workers in this field of research.

#### SUMMARY.

1. The presence of an endophytic mycorrhiza has been established in the roots of *Lolium temulentum*, L., *Lolium perenne*, L., *Lolium multiflorum*,

<sup>1</sup> Bernard looked upon the presence of endophytic forms as a general character, rather than one peculiar to each species, and as he, at the time, could not obtain South American plants, he worked with *Solanum dulcamara*, L., and described a mycorrhiza for this form (4). Madame Noel Bernard and M. J. Magrou, in an appendix to this paper, described a similar mycorrhiza for *Solanum Maglia*, Schlecht, a South American wild form, which of all *Solanums* living in a wild state most nearly approaches the cultivated potato, and which Darwin considered to be the wild form of *Solanum tuberosum* (L.). *S. Maglia*, however, when bred under cultivated conditions, loses its mycorrhiza, but it is possible to re-establish it experimentally. This fact was thought to furnish a new argument in support of Bernard's theory relative to the role of symbiosis in the tuberization of the potato.

Lam., and *Lolium subulatum*, Vis., and a cytological examination of infected roots has been made in the case of Darnel.

2. The occurrence of a mycorrhiza in this grass supports the suggestion, set out in a previous paper, that the evolution in *Lolium* has been along a line similar to the *Calluna* type; however, no work has been attempted to connect the fungus present in the *Lolium* grains with the perpetuation of a mycorrhizal association.

3. The mycorrhiza of *Lolium temulentum* belongs to Gallaud's first series, for the hyphae, intracellular in the first root-layers, become both inter- and intra-cellular in the deeper tissues.

4. Entry into the root is accomplished, either through root-hairs, or directly through the epidermal cells, and it is accompanied by spiral looping in the lumen of the cells of the first and second layers.

5. Arbuscules and sporangioles are formed in the more internal cortical cells.

6. When the association is young and the sporangioles are not yet mature, the hyphae of the outer cells are packed with oil, which stains black with the osmic acid after treatment with a bichromate solution.

7. As the sporangioles reach maturity, the oil is transferred to them, and the hyphae supporting these organs appear in the sections as empty and collapsed. At the same time the sporangioles increase in size and they become uneven in outline owing to the contained fat, the prominences corresponding in position to the fat globules inside the sporangiole wall.

8. Vesicles are formed, both in the intercellular spaces and occasionally in the interior of the cell. At an early stage of development they are packed with fat, which is later given up to the host plant.

9. In old roots gathered during late October and November the sporangioles, by their disruption, had given to the higher plant the fat or oil globules, which now appear free in the cell-cavity.

10. Although these homogeneous globules do not stain black with osmic acid after bichromate, they have, nevertheless, been proved to be fat globules. Some change in the constitution of the hyphal fat apparently occurs before it is liberated into the host-cell.

11. The fat globules are removed from the cells where they first appear, and are apparently used by the grass in the formation of the inflorescence and developing grain. At the same time, the remnants of sporangioles, hyphae, &c., become resolved into a residual inert mass, which remains in the cell probably till the death of the root. The changes described are accompanied by signs of great nuclear activity, evidenced by increase in size, hyperchromatophily, and lobing of that body.

12. The fat globules found in the *Lolium* cells are compared with the homogeneous globules figured and described as nitrogenous in character by Kusano for *Gastrodia elata*. The similarity between the two bodies sug-

gests that the exchange process, which is revealed by a cytological examination of the *Lolium* roots, is closely related to that taking place in this Orchid and is concerned with complex carbon-containing food materials rather than with nitrogenous substances.

13. A critical discussion of the earlier work on mycorrhiza, more particularly that dealing with the physiological relations between the two forms, discloses the fact that the most generally accepted ideas of this relation are those connecting it with nitrogen fixation without any real evidence that such is the case.

14. The demonstration of many infecting strands, together with the appearance of fat, firstly in the conducting and travelling hyphae of the root, with its subsequent removal to the sporangiole, and then to the host-cell, accompanied by collapse and shrivelling of the fungal mechanism, have led to the conclusion that a metabolic exchange takes place *from the fungus to the higher plant, with the result that the latter obtains a supply of fat or oil.*

15. Knudson's results with the non-symbiotic germination of Orchid seeds are examined in the light of this conclusion and fresh support is gained for it.

16. The idea that the exchange is carbonaceous rather than nitrogenous in character is also compatible with Bernard's suggestion of the relation between tuberization in plants and the presence of endophytic mycorrhiza.

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# EXPLANATION OF PLATES I-III.

Illustrating Dr. E. I. McLennan's paper on the Endophytic Fungus of *Lolium*.

All figures have been drawn with the aid of the camera lucida.

## PLATE I.

Fig. 1. A tangential longitudinal section of the cortex of a root of *Lolium temulentum*, L., in the third region.  $\times 625$ . The mycorrhizal association was at a young stage; the travelling hyphae are packed with fat or oil, which stains black with the osmic acid of Flemming's solution. They are seen running through the cells; the majority are supplying sporangioles at a different level. The sporangioles figured are young, and in most cases are not showing their connexion with the hyphal filaments. These are better demonstrated in a radial section. The cell contents at this stage do not include free fat globules.

*h* = hypha travelling to cells at another level packed with fat and appearing black in the section, which was stained with fuchsin-iodine green; *ch* = hypha constricted in passing through wall; *n* = nucleus of host-cell; *dn* = distorted nucleus; *nl* = nucleolus; *pp* = cytoplasm; *sp* = immature sporangioles; *c* = cortical cell of third region.

## PLATE II.

Fig. 2. A vesicle showing a much vacuolated structure and beginning to collapse in the root tissue.  $\times 625$ . *n* = nucleus; *w* = wall; *vac* = vacuole.

Fig. 3. An intercalary vesicle formed in an intercellular space.  $\times 625$ . *iv* = intercalary vesicle; *is* = intercellular space.

Fig. 4. Vesicle with spore-like bodies from a Darnel root.  $\times 625$ . *sp* = spore-like bodies; *rw* = ruptured wall.

Fig. 5. Infection of the root through the penetration of a root-hair.  $\times 625$ . *rh* = root-hair; *h* = hypha running through hair; *a* = abortive branch in epidermal cell; *e* = epidermal cell; *th* = travelling hypha; *c* = cortical cell.

Fig. 6. A single cell of the third region before the disruption of the sporangioles. Note the absence of fat in the cell-cavity.  $\times 1,050$ . *h* = intracellular hypha with reduced contents, *eh* = empty and shrivelled hypha, the fatty content has been transferred to the sporangiole; *sp* = sporangiole; *fs* = fat in sporangiole; *n* = nucleus of host-cell.

Fig. 7. A single cell of the third region after the disruption of some of the sporangioles, others still intact in the same cell.  $\times 1,260$ . *sp* = intact sporangiole; *rsp* = ruptured sporangiole; *fg* = fat globules of large dimensions lying free in the cell-cavity; *sh* = shrivelled and empty hypha; *pp* = cell protoplasm.

Fig. 8. A single cell of the third region after the disruption of the sporangiole; the fat globules are fairly uniform and small in size. (A cell of a communicating strand of the middle cortex.)  $\times 1,050$ . *h* = hypha with fatty content; *eh* = empty hypha supplying cell; *rsp* = ruptured sporangiole; *fg* = fat globules; *c* = cortical cell of third region.

Fig. 9. A cell of the third region after the removal of most of the fat and the formation of the residual mass.  $\times 750$ . *th* = travelling hypha on its way to supply another cell not yet empty of its contents; *f* = fat; *eh* = empty hypha enclosed in granular mass, which bore the sporangioles of this cell; *fg* = fat globules not yet removed from cell; *rm* = residual mass resulting from the digestion of the sporangiole walls, &c.

Fig. 10. Portion of a transverse section of a Darnel root in the region of the endodermis.  $\times 750$ . *en* = endodermis; *is* = intercellular space; *h.is* = intercellular hypha; *fg* = fat globule; *rm* = residual mass; *eh* = empty hypha.

## PLATE III.

Fig. 11. A transverse section of a rootlet of Darnel of approximately 0.75 mm. diameter to show the distribution of the mycorrhiza in the root tissues.  $\times 103$ . *e* = epidermis; *o.c.l.* = outer cortical layer; *l* = lacuna; *s* = stele; *en* = endodermis; *px* = protoxylem; *fc* = fungal cells. (Distribution of the fungus is indicated by shading.)

Fig. 12. Section of a Darnel root to show infection brought about by the direct penetration of an epidermal cell.  $\times 625$ . *e.* = epidermis; *p.h.* = penetrating hypha; *b.* = branch; *t.h.* = travelling hypha; *f.* = fat blackened with osmic after bichromate.

Fig. 13. Outer root-cells of Darnel.  $\times 625$ . *e.* = epidermis; *h.* = hypha (empty); *n.* = nucleus.

Fig. 14. Outer root-cells of Darnel uninfected.  $\times 625$ . *e.* = epidermis; *c.* = cortical cell with cytoplasm; *n.* = nucleus. (Cells are adjacent to those figured in Fig. 4.)

Fig. 15. Cells of Darnel root in outer cortical region to show travelling hyphae.  $\times 625$ . *c.* = cortical cell of second region; *c.h.* = constricted hypha; *h.s.* = hypha in intercellular space; *i.s.* = intercellular space; *f.* = fat.

Fig. 16. Cell of Darnel root from second region.  $\times 625$ . *h.* = travelling hypha; *f.h.* = fat in hypha; *f.g.* = fat globule in cell cavity blackened with osmic acid after bichromate.

Fig. 17. An intercellular vesicle in a young condition.  $\times 1,050$ . *v.* = vesicle; *i.s.* = intercellular space; *n.* = nucleus in vesicle; *c.* = cortical cell of second region; *d.c.* = distorted cell owing to pressure of the vesicle.

Fig. 18. A young sporangiole formed from an intracellular branch of an intercellular filament.  $\times 1,050$ . *w.* = wall of cell; *h.s.* = intercellular hypha; *sp.* = sporangiole; *c.* = cytoplasm.

Fig. 19. Sporangiole showing contained fat.  $\times 1,050$ .

Figs. 20 and 21. Sporangiole from cells of third region.  $\times 1,260$ . *sp.* = sporangiole; *f.g.*<sup>1</sup> = fat globule; *f.g.*<sup>2</sup> = fat globule free in cell; *r.w.* = wall broken artificially, through which fat globule has escaped.

Fig. 22. Cells of the third region just prior to the bursting of the sporangioles. The cell contents at this stage include the nucleus, cell cytoplasm, shrivelled hyphae, and intact sporangioles enclosing the fat about to be liberated into the cell lumen.  $\times 1,050$ . *n.* = nucleus; *pp.* = protoplasm; *i.s.* = intercellular space; *e.h.* = empty hypha; *sp.* = sporangioles; *c.* = cell of the third region.

Fig. 23. Portion of the contents of a cell of the third region at the exchange stage, showing an intact and burst sporangiole.  $\times 1,260$ . *h.* = hypha; *sp.* = intact sporangiole; *b.sp.* = burst sporangiole; *f.g.*<sup>1</sup> = free fat globules; *f.g.*<sup>2</sup> = fat globules seen through wall of intact sporangiole.

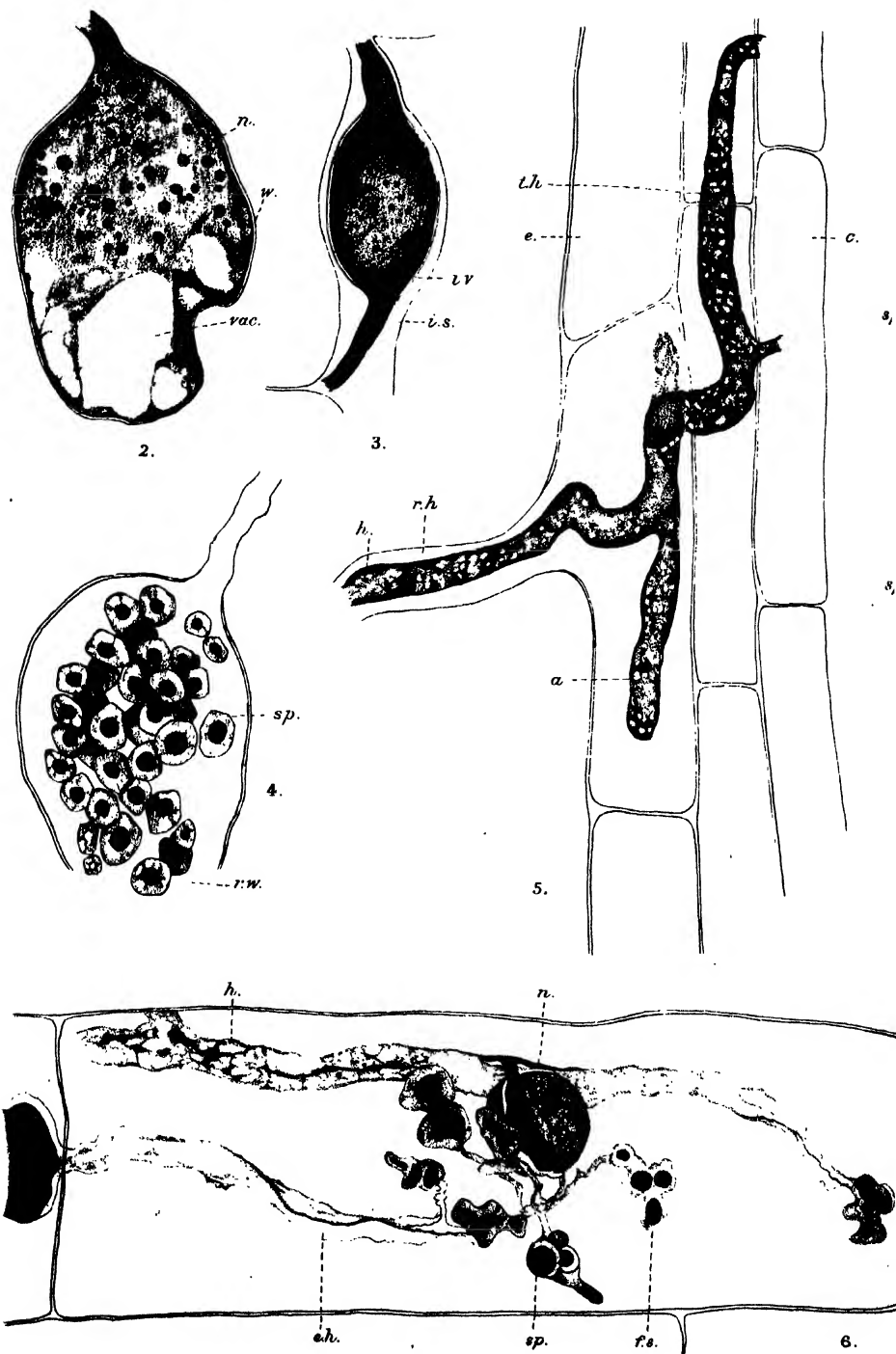
Fig. 24. A cell of the third region after the removal of the fat globules, showing the structureless residual mass crossed by the remnants of travelling hyphae which have supplied other cells of the same region.  $\times 625$ . *n.* = nucleus; *n.l.* = nucleolus; *r.m.* = residual mass; *e.t.h.* = empty travelling hypha; *f.* = fat; *c.* = cortical cell; *i.s.* = intercellular space.



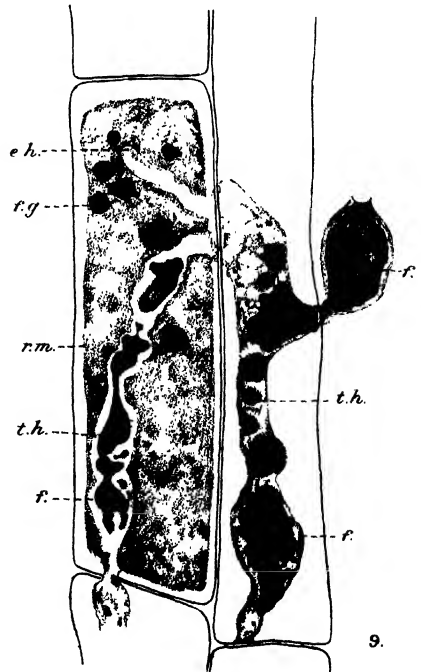
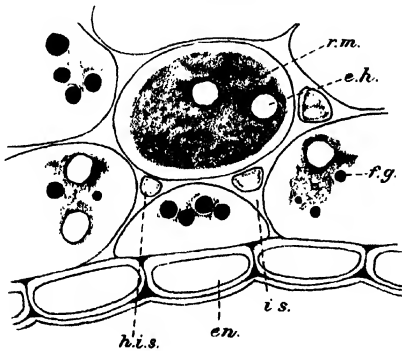
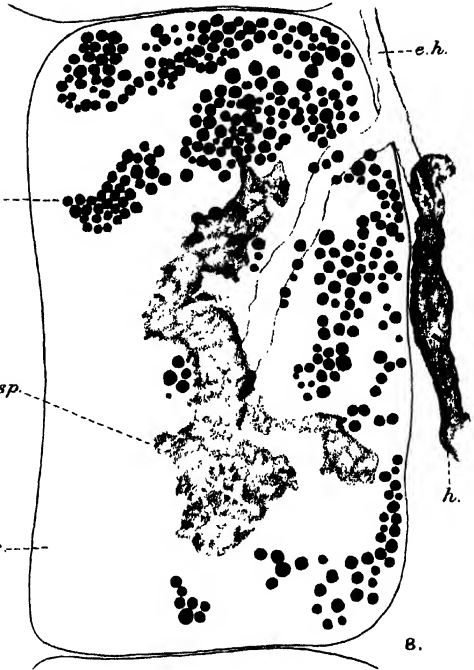
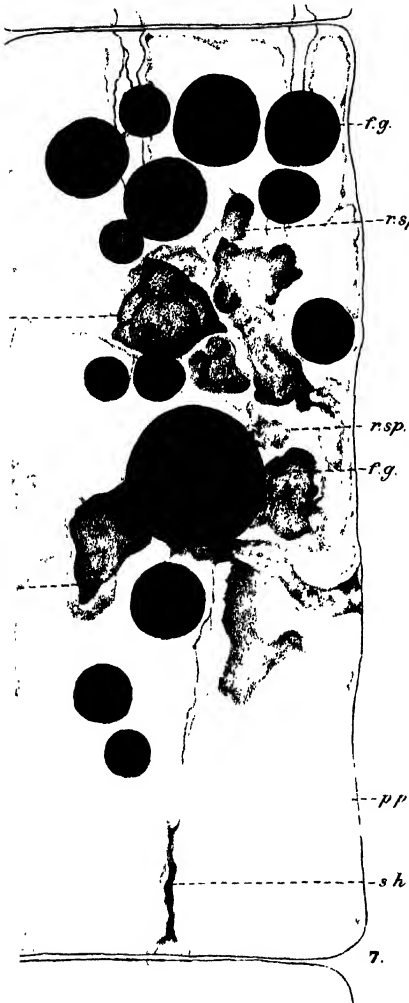
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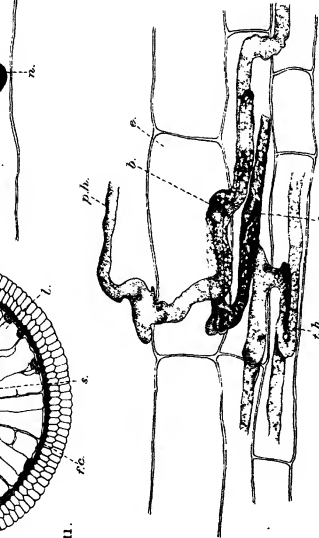
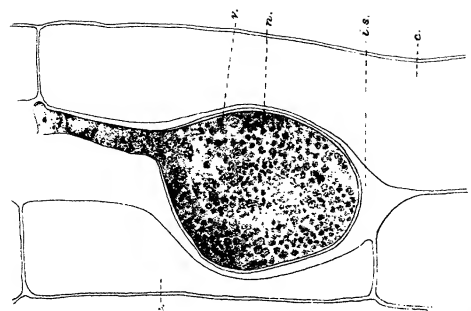
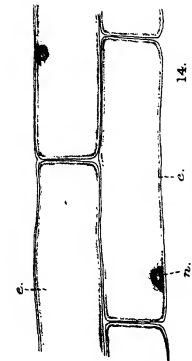
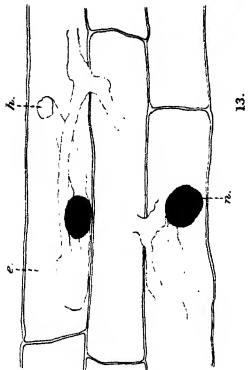
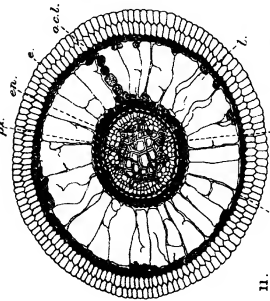
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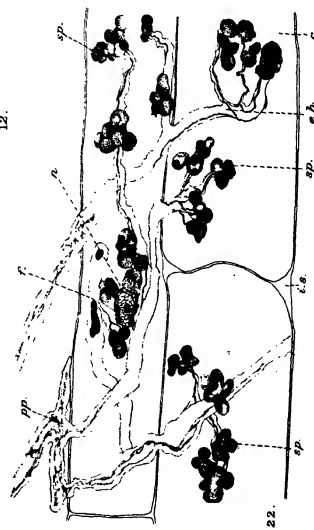


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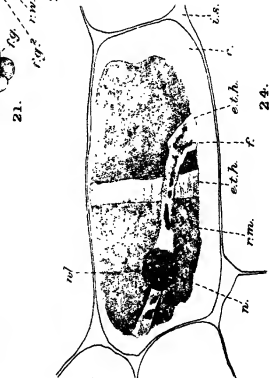




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McLENNAN—MYCORRHIZA OF LILIUM.

Ruth, London.



# The Fungi of Stigmatomycosis.

BY

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With Plates IV and V.

THE purpose of this paper is to discuss the characters of four species of fungi, namely (A) *Spermophthora gossypii*, gen. et sp. nov., (B) *Eremothecium cymbalariae*, Borzi, (C) *Nematospora gossypii*, sp. nov., (D) *Nematospora coryli*, Peglion, of common occurrence in the British West Indies in connexion with stigmatomycosis of fruits, a type of affection in which fruits externally sound are found to be internally infected as a result of punctures made by plant-feeding bugs of the sub-order Heteroptera. From recent evidence it appears that the *Nematospora* species at least have a wide distribution, especially in the tropics.

Borzi described *Eremothecium cymbalariae* in 1888 from the dry capsules of *Linaria cymbalaria* in Italy.

V. Peglion (1) described *Nematospora coryli* in 1897 from mouldy hazelnuts, also in Italy.

G. Arnaud records the finding of *E. cymbalariae* in 1906, 1907, 1911, and 1912 in the dry fruits of *Cachrys laevigata* in the garden of the École d'Agriculture of Montpellier, France. Arnaud describes and figures sporangia and spores which he is inclined to regard as also pertaining to *Eremothecium*, in association with which they were found, but of which he recognizes the close resemblance to *Nematospora*. There can be little doubt, in view of later experience, that he was in fact dealing with a double infection.

W. Nowell in 1915 and 1916 published preliminary accounts of an internal disease of cotton bolls in the Lesser Antilles, associated with the occurrence at different times and places of the four fungi enumerated above.

S. F. Ashby in 1915 published a note of his independent discovery in cotton bolls in Jamaica of two of the species, A and D.

A. Schneider announced in 1916 the discovery of a parasitic Saccharomycete in an imported tomato in California. In a second note, published in 1917, he recognized the affinity of his fungus with *Nematospora coryli* and described it as *N. lycopersici*, giving its habitat as 'nearly ripe fruit of *Lycopersicum esculentum*, Southern California, Cuba, and Mexico'.

Nowell in 1917 published a preliminary description, with figures, of the four species, which were provisionally designated A, B, C, and D in the order given above. The close resemblance of B to *Eremothecium cymbalariae* and of D to *Nematospora coryli* was recognized. In subsequent papers Nowell recorded the occurrence of these fungi in a wide range of fruits, always associated with bug punctures, and described a series of experiments which resulted in the conclusion that natural infection is dependent on bug punctures and that the infecting organism is carried by the bugs themselves.

S. A. Wingard in 1922 described as *Nematospora phaseoli* a fungus occurring in spots on Lima beans and cow-peas in Virginia, U.S.A. The published photographs correspond with the appearance of infections of these seeds commonly seen in the West Indies, but no association with insect punctures is mentioned.

H. A. Lee published in 1924 an account of a dry rot of citrus fruits in China, Japan, and the Philippines, caused by infection with a species of *Nematospora* regarded by him as probably identical with the West Indian form (D) described by Nowell.

The present writers in 1924 identified as species C a fungus held in culture at the Imperial College of Science, London, obtained from cotton bolls grown in Uganda, and have more recently seen the same species in cotton bolls sent as examples of a heavy infestation experienced in Nyasaland.

#### SPECIES A. *SPERMOPHTHORA GOSSYPHII*, GEN. ET SP. NOV.

##### *Primary mycelium.*

##### (a) *Macroscopic.*

Develops well on sweet potato slabs and potato agar with 1 per cent. saccharose as a low white spreading surface growth of hyphal character which may show concentric zonation; no change in colour of potato and no perceptible diastatic action.

(b) *Microscopic.*

Hyphae non-septate, multinucleate, branching, dichotomous, plasma streaming (as in *Pythium*), diameter  $5\ \mu$ . Sporangia develop on potato in a day, in pure culture usually arising subterminally, by swelling of hyphae and septation at both ends of the sporangium. The apical part of the hyphae beyond the sporangium, which may be one to three times the length of the sporangium and simple or bifurcated, makes no further growth. Sporangial enlargement begins before septation is initiated. The septa are formed by a convex ingrowth from the wall, plasmatic connexion being maintained through a central canal which disappears in the mature septum, which is plug-like and of low refringency. No cellulose reaction occurs with iodine-sulphuric acid and chlor-zinc iodine. In the early development of the sporangium glycogen is abundant in the hyphae and enlargements, but rapidly lessens as spores are initiated and is absent from mature sporangia. Terminal sporangia with one basal septum have been observed in nature in cotton bolls and tomato fruit, and occasionally on asparagin-saccharose agar. Sporangia are straight as a rule, but may be curved (comma-shaped), or with double curvature (spirillum-shaped); they are narrow, fusiform, rupturing at any part when mature. Terminal sporangia are cylindrical with broadly rounded apex and narrowed base. The sporangia are very variable in size:  $75\text{--}110\ \mu$  by  $10\text{--}17\ \mu$ ; terminal sporangia in tomato up to  $110$  by  $20.5\ \mu$ .

*Spores.*

The arrangement and number of spores in the sporangium are indefinite; the spores are hyaline, continuous, needle-shaped, in side view convex-linear with acute apex and narrowing to a rounded base; they are widest above the middle. A refringent rib-like thickening of the wall extends beyond the apex of the spore body as a fine point and terminates at the middle; it may be dorsal or ventral in position; the contents of the spore are uniform, finely granular; germination takes place from any part by germ-tube, which at first branches behind the tip, the mycelium developing later by normal dichotomy. Spores  $18\text{--}21$  by  $2\text{--}2.5\ \mu$ .

*Secondary mycelium.*

In pure cultures on potato slabs 2–3 weeks old the primary growth shows scattered white flecks due to development of a secondary growth. This is a simply branched non-septate mycelium of limited development, bearing minute terminal oval sporangia containing a restricted number of hyaline, continuous, cylindrical, distinctly curved spores with rounded ends. Observation has always shown that the secondary mycelium arises from the fusion of two or at times probably more primary spores. These spore

fusions and the development of secondary sporangia have been followed in hanging drops of tap-water. The mycelium may grow out from a hyphal bridge connecting two spores or from any part of the conjugated spores. The spores lose their contents, which pass into the mycelium.

Glycogen is present in the hyphae and in the developing sporangia, but disappears as the spores are formed. The presence of this secondary growth on the primary growth on potato can consequently be readily detected by treatment of scrapings with iodine in potassium iodide. The number of spores in the minute secondary sporangia has several times been seen to be eight, and less frequently twelve. Some, however, may not develop to maturity. The spores escape by rupture or solution of the wall of the sporangium and remain for a time held together in a bundle by slime. They stain deeply and uniformly with gentian violet. In diluted wort or wort agar they swell up considerably and germinate by one or more hyphae, which branch at first irregularly, later by regular dichotomy, and develop primary sporangia and spores. It was at first considered that this secondary spore-forming growth might be due to a parasite, but it has developed regularly from single primary spore cultures, and the primary mycelium and sporangia have developed from single secondary spore cultures. Single spore colonies were obtained by stroking suspensions of spores on plates of corn-meal agar to which 0.1 of its volume of wort was added. Isolated colonies developing clearly from one spore were marked after eighteen hours, and a day later were cut out and transferred to potato.

The secondary sporangia measure 5.7.5 by 3.5-4  $\mu$  (in the case figured 10.5 by 4). The spores are mostly 4.5-6 by 1.3  $\mu$ . On potato and other media the hyphae of the primary mycelium are frequently studded with bud-like outgrowths which have not been observed to separate or develop farther.

#### *Distribution.*

This species was heavily predominant in the bolls of Sea Island cotton in St. Vincent in 1916-17, at times during the crop season when the plants were heavily infested with cotton stainers (*Dysdercus* spp.). It has also been recorded from cotton bolls in Jamaica, Nevis, Montserrat, the Grenadines, and Trinidad. It has been found in tomato fruits in St. Vincent and Trinidad, and in the seeds of cow-pea in St. Vincent.

#### *Diagnosis.*

*Spermophthora*, nov. gen.

Mycelium siphonaceous, multinucleate, branching dichotomously. Sporangia solitary, apical or sub-apical; in the latter a plug-like septum occurs at both ends, formed by ingrowth from the wall. Spores non-motile, indefinite in number and arrangement, escaping by rupture of the sporangium at any part. The spores germinate by germ-tubes which (1)



develop into the primary mycelium or (2) fuse in pairs by short germ-tubes and give rise to a simply branched, secondary, non-septate mycelium bearing terminal sporangia containing a restricted number of spores, probably eight or a higher multiple of four, which on germination develop into mycelium of the primary form.

*Spermophthora gossypii*, nov. sp.

Hyphae  $5\mu$  in diameter in culture, but up to  $8\mu$  in the tissues of the host. Sporangia fusiform and linear, or comma- or spirillum-shaped,  $75-110\mu$  in length by  $10-17\mu$  in diameter when sub-terminal,  $20.5\mu$  in diameter when apical. Primary spores hyaline, convex-linear, broadest above middle, apex acutely pointed and narrowing to a rounded base. A refringent rib-like thickening which may be dorsal or ventral extends beyond the apex as a short spicula and terminates at the middle of the sporc. Spores  $18-21$  by  $2-2.5\mu$ .

Secondary sporangia cylindric-oval, usually  $5-7.5$  by  $3.5-4\mu$ , rupturing at any point when mature. Secondary spores usually eight or twelve, rarely more, in the sporangium, remaining attached for a while when liberated; hyaline-cylindric, curved, with rounded ends:  $4.5-6$  by  $1.3\mu$ .

Parasitic on the lint and in the seeds of *Gossypium* spp. in Jamaica and the Lesser Antilles, in the fruit of *Lycopersicum esculentum* in St. Vincent and Trinidad, and in the seeds of *Vigna catjang* in St. Vincent.

SPECIES B. *EREMOTHECIUM CYMBALARIAE*, BORZI.

*Growth.*

(a) *Macroscopic.*

Develops rapidly on sterile potato slabs and potato agar with 1 per cent. saccharose or glucose. On potato the growth is at first very similar to that of *Spermophthora*, but more vigorous and with more aerial mycelium. After two weeks or more it begins to turn grey and becomes folded or wrinkled, forming a tough plectenchyma with a pilose surface. The potato early acquires a grey tint.

(b) *Microscopic.*

*Mycelium.*

Hyphae non-septate, multinucleate, fine ( $3-3.5\mu$  diameter), branching by regular dichotomy in young, actively growing cultures. In older cultures the hyphae give rise to lateral buds, single or in chains which do not separate readily; they may grow out into hyphae of the normal character while attached to the parent hypha or after separation from it. In old cultures hyphae which appear void of contents may show septa at intervals.

*Sporangia.*

Terminal, or in culture more frequently sub-terminal, by an enlargement of the hypha behind its tip. The sporangium is cut off by one septum at its base, which begins to form after swelling has begun by ingrowth from the wall as in *Spermophthora*, hence plug-like. Glycogen abundant, disappearing as spores are initiated. The sporangia are sometimes bifurcated from the middle or near the apex. The dimensions of the sporangia in cultures are 4.5–5.5 by 1.4–1.9  $\mu$ , including the septum, which is 3.6  $\mu$  thick.

The spores are forty or more in number, tapering to a sharp point at one extremity, blunt and with a more or less angular bend at the other. They occur in a doubly conical bundle formed of two symmetrical groups with the blunt ends interlocked, recalling the appearance of a mitotic spindle. The spores measure 1.5–1.7 by 1.8–2.2  $\mu$ .

*Distribution.*

In the West Indies found in cotton bolls in St. Vincent, Montserrat, Tortola (Virgin Islands), and Nevis, occurring in 89–95 per cent. of the infections in parcels of bolls received from the two last-mentioned islands early in 1918. Fairly common in tomato fruits in the St. Vincent Experiment Station in 1917.

SPECIES C. *NEMATOSPORA GOSSYPHII*, NOV. SP.*Growth.*(a) *Macroscopic.*

Grows freely on sterile potato blocks and on potato agar containing 1–2 per cent. saccharose or glucose.

On potato there is at first a spreading, moist, adpressed hyphal growth with short, pointed, matted, hair-like outgrowths over the surface. After a week scattered pustular dots appear, which unite, forming a folded, vermiform, firmly gelatinous plectenchyma. The hue of the potato remains unchanged, and there are no indications of diastatic action after three weeks.

On potato agar containing saccharose there is at first a spreading, adpressed hyphal growth which changes to a moist, translucent, dirty white, folded, and wrinkled plectenchyma which can be readily peeled off the smooth surface of the agar. In wort the growth assumes the form of mycelial flocks in a clear liquid.

(b) *Microscopic.*

In young actively developing cultures this species grows as a dichotomously branched multinucleate mycelium. The fusiform sporangia are developed by enlargement of successive segments between the septa which

form almost immediately behind the growing-tip, so that the hyphae are converted at an early stage into chains of sporangia. The segments before and during enlargement contain 1-4 nuclei, usually two, three, or four, equidistant; more may be present in fully developed sporangia, but their behaviour just preceding spore initiation has been found difficult to determine. Glycogen is abundant during development, but disappears as spores are formed. As the sporangia approach maturity the hyphae tend to break up by splitting through the septa, the free ends of the sporangia becoming rounded off.

The spores are arranged in bundles with intertwined appendages precisely as in *N. coryli* and their structure and staining reactions are quite similar. The angular expansion or transverse ridge above the middle is, however, obscure or at times apparently wanting in the present species. The number of spores in the sporangium is usually greater than in *N. coryli* and also more variable. It is always a multiple of four. The number of spores in twenty sporangia from a culture on potato was observed to be:

once	.	.	.	.	.	32
once	.	.	.	.	.	24
twice	.	.	.	.	.	20
five times	.	.	.	.	.	16
eight times	.	.	.	.	.	12
three times	.	.	.	.	.	8

The number is most frequently twelve in two bundles of six. Occasionally only four are present. Some long sporangia may contain more than two bundles of spores. As an example, sporangia are not infrequently seen with two bundles of four spores and two bundles of two, or twelve spores in all. The composition of the medium may influence the size of the spores and length of the appendages. In slightly acid peptonized cane juice of about 10° Balling the spore body measured 32-37  $\mu$  in length by 2.5  $\mu$  in width, with appendages twice as long, while on potato the length of the body ranged from 24 to 32  $\mu$  by 2-2.5  $\mu$  in width with appendages 100  $\mu$  or more in length.

In germination a globular expansion arises just anterior to the middle of the body of the spore and gives rise to one or more germ-tubes. In hanging drops of corn-meal agar containing a little wort the hyphae are at first without septa and branch from behind the tips. They bifurcate as they lengthen and septa are formed. The first septum develops 150-200  $\mu$  behind the growing-tip or tips and they are then formed at fairly regular intervals of 50-70  $\mu$ . The septum develops as a ring of convex ingrowth from the wall, gradually occluding the hypha until all plasmatic connexion is severed. The septa may be as much as 6  $\mu$  in thickness and of low refringency like vacuoles, so that their position is difficult to make out in

living hyphae. At an early stage of growth lateral buds are developed on the hyphae singly or in short chains; they fall away and each may germinate by a tube which grows out into the normal bifurcated mycelium. The species just described differs therefore from *N. coryli* in vegetative habit. The hyphal character is much more pronounced and the budding yeast-like character correspondingly reduced. In the dichotomously branched mode of growth it resembles *Spermophthora* and *Eremothecium*, but appears septate owing to the early development of sporangia, whereas their mycelia remain siphonaceous. The older growth on potato closely resembles that of *N. coryli*, but it is firmly gelatinous and not pasty.

#### *Distribution.*

In cotton bolls in Montserrat, Antigua, Nevis, St. Vincent, the Grenadines, and Trinidad; occurring in 90 to 100 per cent. of infections in parcels of bolls from Montserrat collected in 1917 and 1918; in seeds of *Datura metel* in Montserrat; in seeds of *Asclepias curassavica* in Trinidad; in cotton bolls from Nyasaland.

#### *Diagnosis.*

*Nematospora gossypii*, nov. sp.

Mycelium multinucleate, dichotomously branched,  $2.5\ \mu$  in diameter. Sporangia developed by septation followed by enlargement of the successive interseptal segments at some distance ( $150\text{--}200\ \mu$ ) behind the tips of the hyphae,  $70\text{--}100\ \mu$  in length by  $8\text{--}12\ \mu$  in diameter. Septa up to  $6\ \mu$  in thickness, developed by ingrowth from wall. Spores  $4\text{--}32$  in two or more bundles, most frequently 12 in two of 6 arranged as in *N. coryli*. Spores similar to those of *N. coryli*, but transverse ridge above middle obscure or wanting. Spore body on potato  $24\text{--}32$  by  $2\text{--}2.5\ \mu$ , appendage  $50\text{--}100\ \mu$ .

Parasitic on the lint and in the seeds of *Gossypium* spp. in the Lesser Antilles and in Tropical Africa, in the seeds of *Datura metel* in Montserrat, and of *Asclepias curassavica* in Trinidad, West Indies.

#### SPECIES D. *NEMATOSPORA CORYLI*, PEGLION.

##### *Growth.*

##### (a) *Macroscopic.*

Development is rapid on sterilized potato slabs and on potato agar; growth is increased on potato agar containing 1 per cent. or more of saccharose or glucose.

On potato a chalky white raised growth is formed which may attain a height of 2 mm. or more; the structure may be columnar, or compact, or flat-topped with eroded flanks and lobed margin. The lower part of the growth, where moisture is condensed, is dull and sodden, with the appearance of melting snow. Examination with a hand-lens after a few days shows

short, pointed, matted, hair-like outgrowths at and near the margin, or, in some cultures, over the whole surface. Finally the growth sinks down to a rather translucent pasty mass with a wrinkled vermiform surface. The young white growth has little cohesion, resembling freshly fallen snow; it diffuses in a drop of water to a cloudy suspension with escape of air-bubbles. The potato block after a few days becomes more or less grey and finally brown. No evidence of diastatic action can be detected after two weeks.

On potato-agar slopes, with or without saccharose, there develops in a day or two an abundant smooth, dirty white, moist, yeast-like growth, with later a narrow hyphal margin. In acid peptonized cane-juice of 10° Balling there is clouding and a somewhat slimy sediment which stirs up to an opaque suspension without gas production. In wort the appearance is similar, but some cultures form flocks in a clear liquid which begins to cloud only after several days. On nutrient agar the growth is slowly spreading and mainly hyphal, becoming yeasty in the centre if a sugar is present. Cultures on potato media and wort develop a yeasty odour.

(b) *Microscopic.*

The young growth on potato blocks consists of (a) oval or spherical budding cells either isolated or in short chains, (b) hyphal filaments of long narrow cells which tend to separate, (c) large narrow oval or cylindrical sporangia, some with developing or mature spores, and bundles of free spores, some of which may be germinating, (d) large numbers of spherical or sub-spherical thin-walled fragile cells 15–25  $\mu$  in diameter; these elements appear as if completely occupied by a single vacuole, but a fine plasmatic reticulum can be seen under a high power.

In hanging drops of corn-meal agar with a little wort added development can be followed. On this solid medium rounded or apiculate oval yeast-like cells 6–12  $\mu$  in diameter give rise to simple or branched chains by budding from both ends and by sub-apical lateral buds in a manner quite similar to many yeasts. A hyphal character develops when the successive buds stretch into long narrow cells constricted at the base, where a distinct transverse septum is formed. Chains of curious flask-shaped cells are frequently developed in this way. As the film becomes exhausted of nutriment the cells become larger and more vacuolate. Development of sporangia accompanies multiplication by budding. They develop by extension of single cells in a chain or by a lateral outgrowth from a cell which is often the mother-cell of the chain. In the definitely hyphal thallus produced under some conditions they arise by lateral outgrowths of cells in the hypha either from the middle of the cell or at one side of the apex, and commonly retain a T-shape or hook-shape when fully developed. During development sporangia may produce both apical and lateral buds.

The sporangia produced in the normal type of growth may, during

development, be naviculate in shape, with a slight angular enlargement at the middle, or be distinctly narrowed at or near the middle. The latter appearance can be seen at an early stage of growth and is somewhat suggestive of conjugation of two adjoining cells in a chain. The suggestion is strengthened by the fact that in staining the films a nucleus occupies the narrow zone. Actual conjugation as described and figured by Schneider has not been seen. It is certain, however, that sporangia are frequently developed from single cells in the absence of any preceding fusion. The vegetative cells and very young sporangia are uninucleate, but the latter usually soon become binucleate, and this condition is retained until they are fully developed. Immediately preceding spore-formation the two nuclei lose their identity and seem to become diffused in the cytoplasm. Metachromatic corpuscles (volutin), oil globules, and glycogen are present in developing sporangia. The long, slender spores are arranged in two bundles at opposite ends of the sporangium parallel with its longer axis. The two bundles are connected by the cohering filamentous appendages, the ends of which are intertwined. It frequently happens that the sporangia are not long enough to allow of a free space between the spore-bundles, so that these overlap to a variable degree. The number of spores in the sporangium is most frequently eight, four in each bundle. Small sporangia, however, frequently develop only four (two in each bundle), or more rarely two. The spores escape by rupture of the sporangial wall at any part, the bundles remaining united for a time by the appendages. The wall of the spores is not easily wetted, as they collect in masses at the surface of a drop of water in which a small fragment of a culture has been diffused. The appearance of the mature spore-body depends on the position in which it is lying. If lying on its side the contour appears flattened convex-linear, the convexity being interrupted by an angular expansion (transverse ridge) above the middle. In dorsal view the outline is nearly fusiform with a slight angular expansion above the middle on both sides. The anterior half of the spore is thin-walled and the contents homogeneous. It tapers to a rounded tip to which is attached a long flexible whip-like appendage thickened towards its base. The posterior half has a thicker, more refringent wall, and the contents show several vacuoles separated by bands of plasma; it terminates in an acute point extending beyond the body as a spicula about  $2\mu$  long. At the junction of the anterior and posterior parts, approximately at the middle of the body and  $2.5\text{--}3\mu$  below the point of the angular expansion, a transverse line marks the abrupt separation of the two regions of differing refringency; being an optical effect it cannot be mistaken for a septum. Both Schneider and Wingard mention and figure a septum at the angular expansion, but none is visible at this point in spores from a culture furnished by Wingard; moreover, the mode of germination negatives its presence at that point. In many living mature spores a clear transverse zone can be

detected immediately anterior to the junction of the two regions. It resembles a vacuole, but is of uniform depth ( $1-1.5\mu$ ) and may be a septum of low refringency. The appearance of developing spores lends some support to this interpretation, as well as the frequent presence in stained films of a fine plasmatic thread crossing the clear zone in maturing spores before they escape from the sporangium, suggesting the manner of development of a broad septum by ingrowth from the wall in the hyphae of *Nematospora gossypii*. The anterior and posterior regions of the spore stain with unequal facility. When suspended in aqueous solutions of gentian violet or methylene blue the anterior part, which bears the appendage, becomes deeply stained before the posterior part shows any staining. When films are stained for a day and then washed with alcohol the anterior part may be decolorized before the posterior region shows any loss of colour. This behaviour suggests that the wall of the posterior region is not readily penetrated either by water or alcohol. An indistinct nucleus is present in the anterior part of the spore. In germination the anterior region swells up near the middle of the spore-body to a sphere which gives rise from one or more points either (*a*) to buds which grow into branched chains of oval cells, so that the spore becomes covered by a yeast-like colony, or (*b*) to short septate hyphae which develop buds at the tips. Occasionally the spherical enlargement develops directly into a sporangium. The posterior region of the spore may increase in refringency during germination, but undergoes no other change. Two strains have been isolated: one from Cauto cotton bolls in Jamaica, and the other from Sea Island bolls, tomato fruits, and the seeds of a variety of plants in the Lesser Antilles. The Jamaica strain is identical with the second in cultural characters, but the sporangia and spores are longer and the appendages relatively shorter. The under-mentioned measurements in microns of living sporangia and spores mounted in water were obtained from cultures of the two strains on asparagin-saccharose agar of the same age. The dimensions given by Peglion for *N. coryli* are also recorded.

	Jamaica strain.	Lesser Ant. strain.	<i>N. coryli</i> .
Sporangia	84-95 × 10-13	54-68 × 10-13	65-70 × 6-8
Spore-body	34-48 × 2-3	30-38 × 2-3	38-40 × 2-3
Appendage	31-48	50-66	35-40
No. of spores in } sporangium	usually 8 in two bundles	usually 8 in two bundles	8 in two bundles

A culture of *N. phaseoli*, kindly supplied by Mr. Wingard, showed no cultural differences from those isolated from cotton bolls and tomato fruits from the Lesser Antilles. The width of the spore given by Schneider for his *N. lycopersici* isolated from tomato fruit imported from the West Indies or Mexico into California is not in agreement with his figures, which indicate a spore not exceeding  $3\mu$  in diameter. His description of *N. lycopersici* is in all other essential respects in closest agreement with the West

Indian form. As the dimensions of the sporangia and spores and the arrangement and number of these in the sporangium given by Peglion are also in close agreement, it seems unnecessary, pending a re-isolation of his species from hazel-nuts from Europe, to regard Schneider's, Wingard's, or the writers' forms as other than strains of *N. coryli*.

#### *Distribution.*

This is by far the most generally distributed agent of stigmatomycosis in the West Indies, having been found in fruits representing sixteen genera and eight orders, while the list could certainly be largely extended by further investigation. The fungus has been met with throughout the Lesser Antilles including Barbados.

To these records should be added Lee's records for China, Japan, and the Philippines, and according to the views above stated, those of Schneider for S. California, Cuba, and Mexico, and of Wingard for Virginia.

The occurrence of the fungus on cotton is usually associated with infestation by the green bug *Nezara viridula*, or by other bugs with a wider range of host plants than is frequented by the cotton stainers (*Dysdercus* spp.), which breed only on Malvaceae and the closely allied Bombacaceae. *Nezara* is at times very abundant in St. Vincent on *Cajanus*, *Dolichos*, *Phascolus*, *Vigna*, and other cultivated genera of Leguminosae, and transfers infection from these plants to neighbouring cotton.

#### *Systematic position.*

The mycologists who have discussed the genus *Nematospora* have formed their conclusions on *N. coryli*, or on the more recently described species which bear so close a resemblance to *N. coryli* that they are regarded by the present writers as probably identical with it. The thallus of *N. coryli* is admittedly typically toruloid, its mycelial forms being developed only under adverse conditions or after long artificial culture. We have in *N. gossypii* a species which, from the close correspondence of its sporangia and of the very special type of spore, cannot be denied admission to the genus, but in which the position as regards the thallus is reversed, its characteristic form, as found in its natural habitat and in healthy cultures, being definitely hyphal, while the toruloid form is assumed only when growth is weak and slow, and is moreover not accompanied by spore production.

The confidence with which *N. coryli* is classed as a Saccharomycete and its sporangia are described as asci must be considerably checked when *N. gossypii* comes under consideration. The latter species will be admitted to be an embarrassing claimant for inclusion in the Saccharomycetes, and the aptitude of *N. coryli* to assume a hyphal form, with a definite type of



sporangium, forms a link which would seem to serve better to withdraw *N. coryli* from its previous association than to bring in *N. gossypii* to keep it company.

The writers feel that more investigation is required, and especially cytological study, before the systematic position and relationships of the genera dealt with can be intelligently discussed. Influenced by the interchangeable occurrence of these fungi in the same diseases, they feel that the links between them, though not at present strong in any direct line, are stronger than any other affinity at present recognizable and may be reinforced by further study. Should their relationship be confirmed, it would seem that a new group will be required for their accommodation.

The extent to which the three genera have salient characters in common is set out below.

*Spermophthora, Eremothecium, Nematospora.*

1. Spores formed within a sporangium originating in the expansion of a hyphal cell, and liberated by the solution of the sporangium wall; capable of germination to form a mycelium direct.
2. Hyphae continuous, except in connexion with sporangium formation; branching by regular dichotomy.
3. Known to occur only as parasites in fruits and seeds, infection appearing to be entirely dependent on introduction by insects of the sub-order *Heteroptera*.

*Eremothecium, Nematospora.*

1. Spores formed in two equal opposed bundles in the sporangium.
2. Spores exhibiting a difference of refringency on the opposite sides of a line of separation near the middle, but without a distinguishable septum, and germinating from a spherical expansion formed on one side of the dividing line.

*Spermophthora.*

Spores conjugating to form a secondary mycelium on which are borne secondary sporangia with a limited number of spores of distinct form.

So far as their characters are known, the principal difference between *Nematospora gossypii* and *Eremothecium* lies in the absence from the latter of the peculiar spore appendages. *Spermophthora* differs more widely, as regards its primary sporangia, by the indeterminate arrangement of the spores and by the form of the spores themselves. The secondary sporangia of *Spermophthora* have the characters necessary for them to be regarded as asci and for the fungus to occupy a position in the Protoascineae. This excludes the primary sporangia in *Spermophthora* from the category of

asci, and the exclusion will extend to the sporangia of *Eremothecium* and *Nematospora* if they are regarded as homologous. Such a relation is admittedly somewhat doubtful on the evidence so far available, but, as already stated, the general resemblance between the fungi on the sum of their characters is suggestive of its possibility.

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#### EXPLANATION OF PLATES IV AND V.

Illustrating Prof. S. F. Ashby and Mr. W. Nowell's paper on the Fungi of Stigmatomycosis.

##### PLATE IV.

##### A. *Spermophthora gossypii*.

Fig. 1. Immature sporangia.

Fig. 2. Mature sporangium.

Fig. 3. Terminal form of sporangium.

Fig. 4. Spores and stages of germination.

Fig. 5. Dichotomy of hyphal tips.

Fig. 6. Hypha showing lateral buds.

Fig. 7. Conjugating primary spores forming secondary mycelium and sporangia.  $\times 1,000$ .

Fig. 8. Fragment of more extensive secondary mycelium bearing sporangia.  $\times 1,000$ .

(Figs. 7 and 8 are from potato culture 2-3 weeks old.)

Fig. 9. (a) Secondary sporangium mounted in water.  $\times 2,000$ .

(b) The same after pressure applied to cover-slip.

Fig. 10. Secondary spores germinating on corn-meal agar with wort.  $\times 1,000$ .

B. *Eremothecium cymbalariae*, Borzi.

Fig. 11. Mycelium with maturing sporangium.

Fig. 12. Sporangium with eroded wall, after pressure applied to cover-slip.

Figs. 13-15. Developing sporangia.

Fig. 16. Bifurcated sporangia.

Fig. 17. Sporangium after discharge of spores.

Fig. 18. Spores and spores germinating.

Figs. 19-20. Development of mycelium from a spore.

Fig. 21. Hyphae in lumen of a cotton fibre.

C. *Nematospora gossypii*.

Fig. 22. Hypha with sporangia in various stages.

Fig. 23. Mature sporangium.  $\times 750$ .

Fig. 24. Mature sporangium with eroded wall.  $\times 750$ .

Fig. 25. Spores.  $\times 1,000$ .

Fig. 26. Spore germinating to form a yeast-like colony.

Fig. 27. Spore germinating to form mycelium.

Fig. 28. En hypha from germinated spore in hanging drop of corn-meal wort agar one day old, showing dichotomy and development of septa. (Stained with aqueous gentian violet.)  $\times 200$ .

Fig. 29. Development of sporangia and buds in hanging drop after two days.  $\times 220$ .

Fig. 30. Cells budded off from mycelium, germinating.  $\times 500$ .

PLATE V.

D. *Nematospora coryli*.

Fig. 31. Fragment from a normal actively growing colony, mounted in water, showing spherical cells, toruloid groups, and development of sporangia.

Fig. 32. Group developed from a toruloid cell in a hanging drop of nutrient solution.

Fig. 33. Spherical and flask-shaped cells on a cotton fibre from a diseased boll.

Figs. 34-35. Abnormal sporangia.

Fig. 36. Contents of a sporangium immediately after release.

Fig. 37. Spore in two positions.

Fig. 38. (a) Ordinary germination of spores.

(b) Germination to form sporangia.

Fig. 39. Mature sporangium developed direct from a spore.

Fig. 40. Hyphae and sporangia developed by growth in tap-water.

Fig. 41. Sporangia in stained film from potato culture one day old.  $\times 600$ .

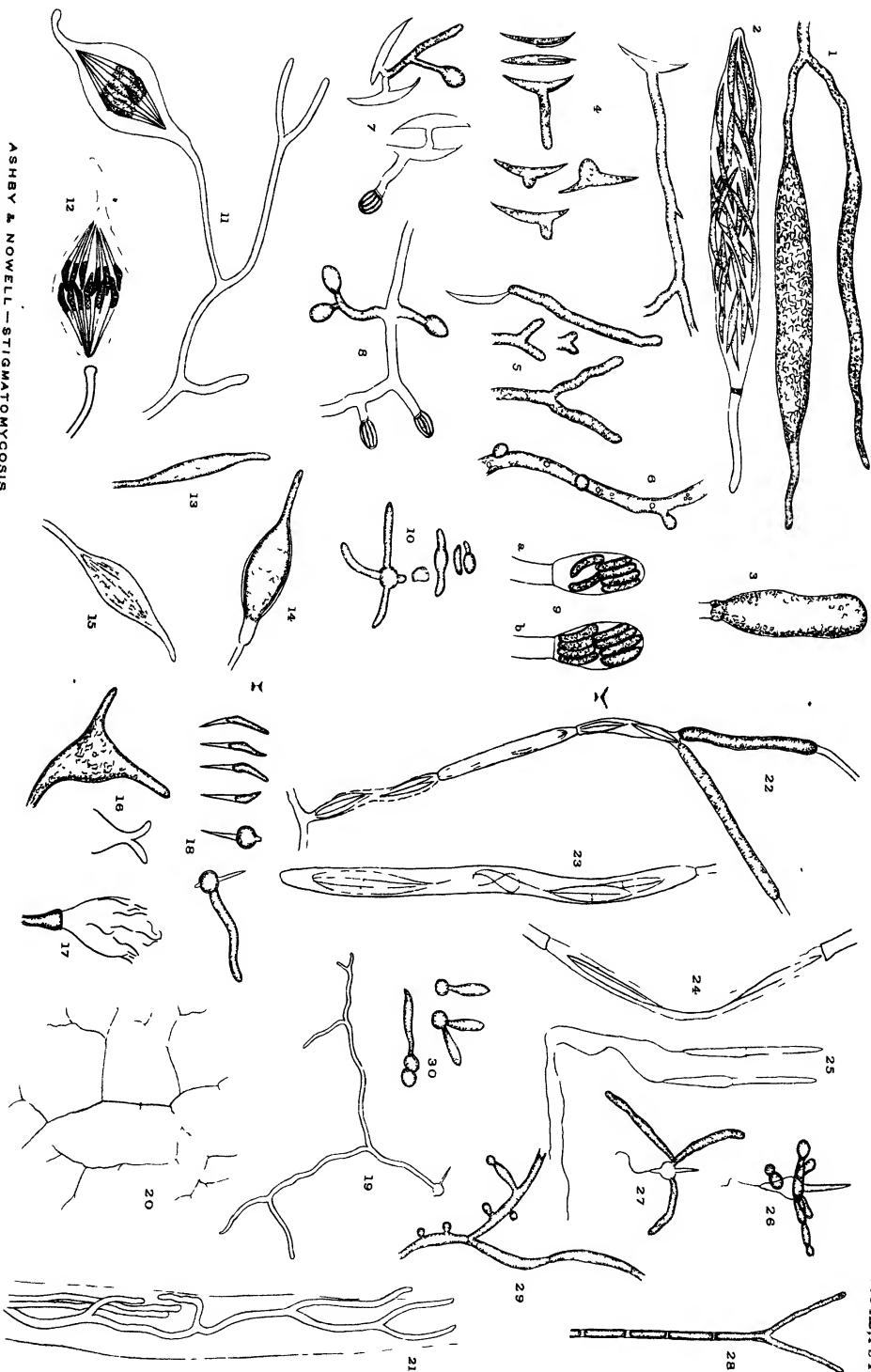
(a) Uninucleate sporangium. (b) Uninucleate sporangium becoming binucleate.

(c) Nuclear division preceding spore formation. (d) Young sporangium suggesting conjugation.

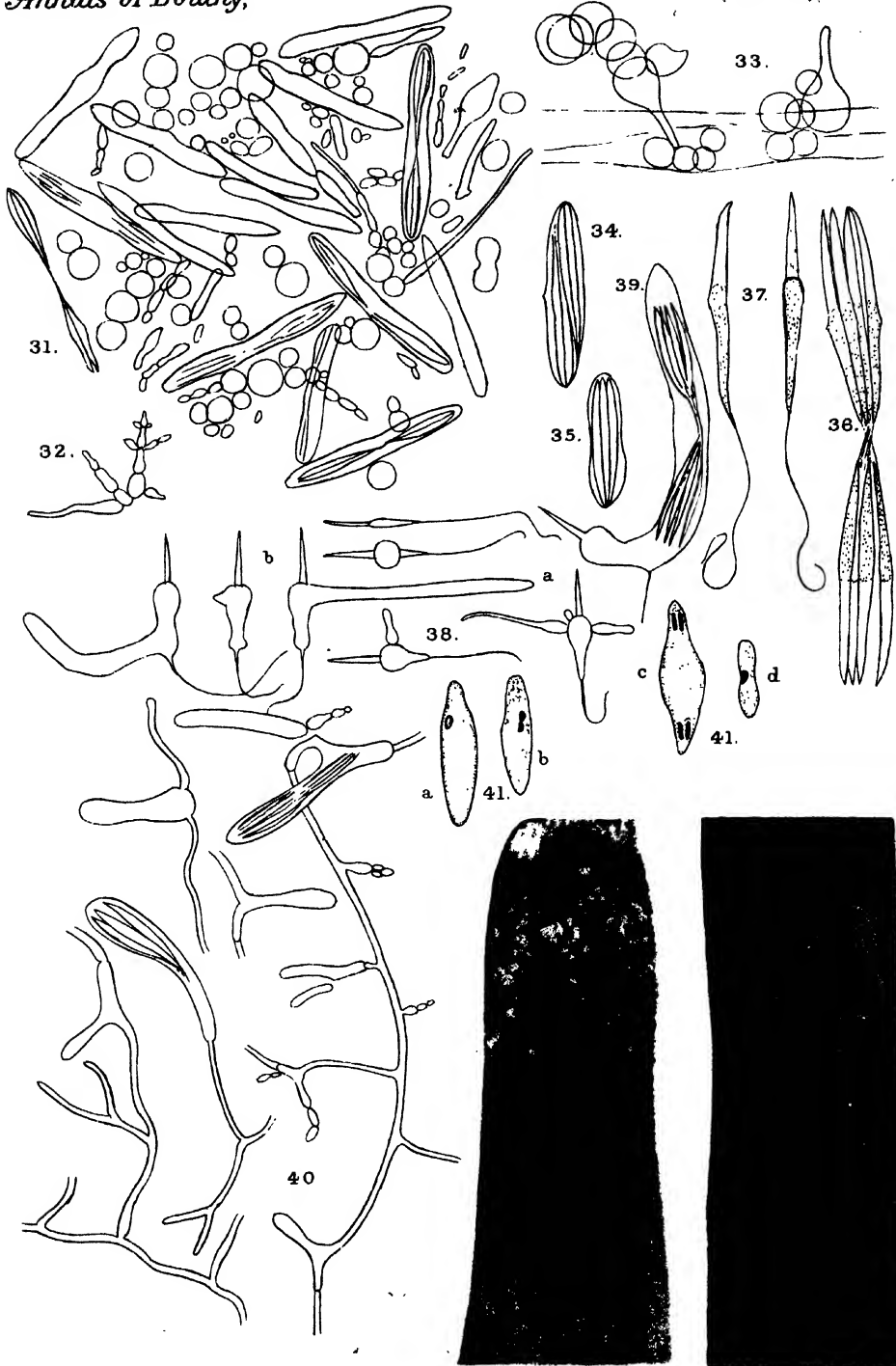
Fig. 42. Culture of *Nematospora gossypii* on potato.

Fig. 43. Culture of *Nematospora coryli* on potato.

(Figs. 42 and 43 are from photographs by Mr. F. W. Urich.)









## Growth Studies.

### V. Factors affecting the Development and Form of Leaves.

BY

W. H. PEARSALL

AND

ALICE M. HANBY.

With three Figures in the Text.

IN the experimental study of the relation between growth and form, one of the most obvious lines of attack is that presented by the variations in leaf form which are so frequently encountered in nature. These variations are often complex, and in many cases might reasonably be attributed to the operation of a number of factors. Little experimental work appears to be available, however, which has aimed at the consideration of these form variations in terms of internal factors affecting the dividing cells. While the present communication does not solve many of the problems which arise, it represents an attempt to state the question of leaf form from a causal point of view, and in terms of recent work on plant growth. The question, as a whole, is considered chiefly in reference to the leaves of Dicotyledons, and our work deals particularly with the palmate type of leaf.

#### *Development of Leaf Form.*

The final form assumed by a given leaf is clearly the resultant of two periods of growth, which may perhaps be contrasted as the period of *initiation* and the period of *development*. In most woody Dicotyledons of temperate climates, the spring leaves are initiated during one growing season and they develop during the subsequent season. We do not propose to deal with the factors which initiate the forms of the different leaf types in



the present paper. We start, therefore, with a more or less specific basis of leaf form as it has been laid down in the bud and examine the modifications which may arise subsequently.

In typical palmate leaves like those of Vine (*Vitis vinifera*), Sycamore (*Acer Pseudoplatanus*), and Ivy (*Hedera Helix*), the young leaf usually appears with its main axis projecting as a continuation of the leaf-stalk. Soon, however, the lamina bends over so that it now forms an angle, usually about  $90^\circ$ , with the petiole. This change in orientation is then followed by a change in form, for the basal lobes now become relatively more prominent in relation to the remainder of the lamina. The fact is illustrated by the following table, in which the length of the subordinate lobes of five-lobed Ivy leaves is expressed as a percentage of the length of the main vein. The figures are averages, twenty leaves having been measured in each class. The change in the orientation of the lamina took place, in this case, when the main vein was about 20 cm. in length.

TABLE I.

*Proportionate Lengths of Lobes of Ivy Leaves during Development.*

Midrib length (M) in mm.	10-20	20-30	30-40	40-50	50-60	+ 60
First laterals per cent. of M.	67.1	70.2	76.0	80.0	83.4	88.0
Basal lobes per cent. of M.	54.9	52.5	59.6	62.0	65.6	70.3

This difference in the relative development of the lobes may be tentatively explained in the following way. In young leaves, when the main vein and petiole lie in the same straight line, the basal veins make a large angle with the main vein, and hence it may be difficult for solutions flowing into the leaf from the petiole to change their direction of movement and flow down the basal veins. This disability is lessened when the lamina makes an angle of  $90^\circ$  with the petiole, because then all the veins make approximately the same angle with the petiole. Hence the alteration in orientation tends to equalize the relative rates of growth in the various lobes, and those at the base become relatively larger. We shall be able to state this problem more precisely at a later stage.

In Horse Chestnut (*Aesculus Hippocastanum*) leaf development proceeds in a somewhat different manner. When the leaf emerges from the bud, the veins of the lateral and main leaflets are parallel to the leaf-stalk and prolong it in the same direction. The leaflets then bend back until they are almost touching the leaf-stalk, and finally are slowly raised until they assume their final position. This is usually a stage in which the main vein continues the line of the petiole, while the lateral veins lie in the same

plane, but make angles of  $45^\circ$ ,  $90^\circ$ , and  $135^\circ$  with the main vein. When the leaflets emerge they are all of somewhat similar length (see below), these proportions being roughly retained during the period of movement, i.e. while the leaflets make similar angles with the leaf-stalk. When the leaflets approach the final position, however, the upper ones become relatively larger.

TABLE II.

*Representative Proportions of Horse Chestnut Leaves at Different Stages  
(as Percentages of Length of Main Vein).*

Stage.	Main Vein.	Lateral 1.	Lateral 2.	Lateral 3.
1. Emerging from bud	100 (25)	96	85	73
2. Movements ceasing	100 (100)	95	83	75
3. Mature	100 (225)	98	74	45

(Averages of ten leaves; figures in brackets give average length in mm.)

In this case, the final position would be such as to make it difficult for solutions to flow readily into the basal leaflets, and their growth is, therefore, relatively retarded.

Entire leaves will not be discussed in detail, since they alter shape very little during development. Apparently the general tendency is for these leaves to lengthen more than they broaden (Arnoldi, 1). The direction of greatest growth thus coincides with that which would be taken by solutions flowing along the leaf-stalk and main vein.

The view of the importance of the relation between the angle made by the chief veins of a leaf and the petiole may be partly justified by simple experiments. If young *Vitis* or *Acer* leaves are clipped so that they are fixed with the main veins in prolongation of the petiole, then their final proportions resemble their initial ones and no relative increase in the basal lobes occurs. Occasionally, leaves may be found in nature in which the youthful condition has persisted.

TABLE III.

*Effect of fixing the Main Vein of a Leaf in the Same Line as the Petiole*

	<i>Acer Pseudoplatanus.</i>			<i>Vitis vinifera.</i>		
	Normal.	Clipped. Finally.	Originally.	Normal.	Clipped. Finally.	Originally.
Main vein	100 (87)	100 (72)	100 (24)	100 (59)	100 (62)	100 (22)
First lateral	88	75	76	85	68	74
Basal lobe	51	32	40	68	53	50

Figures give length of lobe as per cent. of main vein. Those in brackets are length in mm.

A variation of this experiment can be carried out by fixing the leaf so that the lamina is bent back to touch the petiole. Under such conditions pronounced growth of the basal lobes results, so that the final proportions in *Vitis* would be for main, lateral, and basal lobes, respectively 100, 100, and 72. Here again the greatest growth occurs in those veins which would most easily receive solutions flowing into the leaf.

These experiments need a certain amount of care. It is advisable to keep the leaves in a similar position to that of the control—preferably horizontal—and to avoid constricting any of the vascular bundles.

The suggestion made above that the angles at which the veins leave the petiole may determine the ease with which solutions will flow into these veins, and hence the rate of growth of the various lobes of the leaf, is, at first glance, an attractive and simple explanation of the facts recorded. It requires, however, further examination, because the flow of liquid into a given set of tubes is determined not only by the angle these tubes

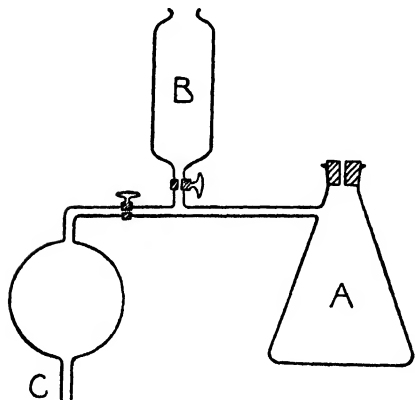


FIG. 1. For description see text.

make with the direction of flow, but also by the rate of flow and the hydrostatic pressure. We have therefore to consider the general problem of water-supply to the lobes of a leaf and also the relation of growth to this factor. Growth also appears to show some direct relation to hydrostatic pressure. It is known that growth will not continue if the water columns in a plant are under tension. This was observed, for example, in Egyptian Cotton by Balls (3), and the fact suggests that we have to deal with a growing system which is normally irrigated by aqueous solutions under pressure.

#### *Hydrostatic Pressure and Growth.*

In order to determine the effect of hydrostatic pressures on leaf growth, experiments were carried out with the apparatus figured (Fig. 1). The shoot of the plant used was placed in A, the cut end being sunk to near the bottom of the flask, so that air bubbles could collect at the top without disturbing the experiment. Water or nutrient solution was added from the reservoir B when necessary. The required hydrostatic pressure or tension was obtained by adjusting a movable mercury column attached to C. Most satisfactory results were obtained by using woody plants, but large seedlings of beans with or without the cotyledons have also been employed. Controls

of similar plants, set up in similar apparatus at the same time, have been used throughout. Rapidly growing leaves are essential. The amount of growth was measured from the base along the midrib of the leaf from a fixed fine mark of Indian ink, using a scale graduated in 0.5 mm. The following results with young Cherry Laurel leaves (*Prunus lauro-cerasus*) are typical of those obtained. As the plants absorb a certain amount of water, the pressures used vary somewhat unless constantly adjusted. In the experiments quoted the pressures or tensions employed varied between 15 and 25 cm. of mercury. They were adjusted to the lower figure at 9.0 a.m., 1.0 p.m., and 5.0 p.m. each day. The twigs used were 30 cm. in length, bearing five leaves, of which the upper one or two were growing rapidly and between 5 and 10 cm. in length.

TABLE IV.

*Effect of Pressures.*

Growth per Day in mm. in successive Days.

I. Leaf No.	1.	2.	3.	
Water	1.0	1.0	1.5	
Increased pressure	2.0	0.5	1.25	
	3.0	1.5	2.0	
	1.0	1.0	2.5	
II. Leaf No.	4.	5.	6.	7.
Increased pressure	2.5	3.0	1.5	1.5
	0.5	2.0	1.5	0.5
Water	0.5	0.5	0.5	1.0
	1.0	0	0.5	1.0
III. Leaf No.	8.	9.	10.	
Water	2.5	2.5	1.0	
	3.5	2.5	1.0	
Reduced pressure	1.25	0	0	
	0	1.0	0	
	0	0	0	

The average growth per day of ten similar leaves left in water during this period of four days was  $1.4 \pm 0.5$  mm. All the leaves grew. Out of three leaves treated with reduced pressures all this time, two did not grow at all and showed no subsequent growth in water, the third grew in the four successive days 2 mm., 0.5 mm., 0, 0. The conclusion to be drawn from these results appears to be that negative pressures stop or markedly reduce growth, while increased positive pressures tend to increase the rate of growth. There is obviously marked variation both in the normal rate of growth and in the response to the pressure conditions applied in the experiment. Essentially similar results have been obtained with *Vicia Faba*, *Acer Pseudoplatanus*, *Aesculus Hippocastanum*, *Fuchsia* sp., *Vitis vinifera*, and *Camellia japonica*. In the last named growth is very slow, and the only effective observation made was that no growth occurs under reduced pressures. On the whole these plants are probably more sensitive to pressure

conditions than are Cherry Laurel leaves. In the case of *Vicia Faba*, wilting was readily produced at the end of May, 1921, by negative pressures of as little as 2.8 cm. of mercury. This obviously tends to confirm the work of Knight (5) and of Maximow and Krasnosselsky-Maximow (7), who find that a very slight reduction of water content during the day-time is sufficient to cause wilting.

The evidence obtained suggests that hydrostatic pressures are necessary to growth, and shows that small negative pressures are sufficient to reduce and stop growth. It is, however, not yet shown that hydrostatic pressures exist in rapidly growing organs. To obtain evidence on this point, rapidly growing leaves have been examined as soon after dawn as possible, the tip of the main vein being cut off with a sharp razor to see if the liquids present are under pressure. Drops of water are exuded in the following cases: *Acer Pseudoplatanus*, *Aesculus Hippocastanum*, *Vitis vinifera*, *Pteridium aquilinum*, *Platanus orientalis*. In other cases there may be no noticeable exudation, but on holding the leaf up to the light the marginal areas near the veins are seen to be suffused with liquid. In these cases, drops of liquids at the ends of veins are not to be expected, since the pressure has apparently found an outlet in the intercellular spaces. Suffusion of this type occurs in *Tilia europaea*, *Fraxinus excelsior*, *Populus nigra*, *Prunus Avium*, and *P. Lauro-cerasus*. It is to be noticed that the large-leaved species examined belong to the first class, while the types showing suffusion had rather smaller leaves. Most of the small-leaved trees belong to a third type, in which neither exudation nor suffusion has yet been observed in the leaves. The species which are for the present left in this class are *Betula pubescens*, *Fagus sylvatica*, *Pyrus Aria*, *P. Aucuparia*, *Alnus glutinosa*, *Salix fragilis*, *S. cinerea*, *Populus alba*, *Ligustrum vulgare*, *Laburnum vulgare*, *Ulmus campestris*, and *Syringa vulgaris*. Opportunities of examining some of these species have been limited, and the list must not therefore be regarded as final. It is also necessary to state that exudation and suffusion have only rarely been observed during the day-time, and it is possibly significant that these occasions were all on warm and damp days. According to our measurements, these leaves rarely show any appreciable growth after the early morning.

There is, of course, apart from the definite cases, considerable evidence that hydrostatic pressures may exist in plants. The evidence as to positive pressures in woody plants is well known, as also is the fact that the highest pressures occur about the time of maximum growth in spring. The data are summarized conveniently in Pfeffer's text-book (10). Exudation from leaf-tips is familiar to most botanists, and it is also known that it occurs most often at night or in moist air. Malin Smith (11) has indicated the extent to which the rapid growth of the giant bamboos is associated with exudation, and both he and Lock (6) found that the humidity of the atmo-

sphere was usually the factor limiting the growth of these plants in Ceylon. Periods of rapid transpiration caused a cessation of growth. The balance of evidence, therefore, permits us to assume that rapid growth is usually associated with positive hydrostatic pressures and that negative pressures prevent growth.

Further inquiry may now be made as to the effects produced in the growing tissues by changing pressure conditions. There are two ways in which positive pressures might conceivably affect growth in length, either (i) by altering the rate of cell division, or (ii) by altering the rate or the extent of cell elongation. Although we were originally tempted to consider the second of these possibilities as the more likely, the evidence available points definitely to alterations of the rate of cell division as the underlying cause. There are well-marked differences in the structure of leaves grown under positive and negative pressures. In the former, the palisade cells are rather smaller in surface view than they are in leaves grown under negative pressure. In addition the areas enclosed by the minor veins (the 'vein islets') are markedly less for leaves grown under positive pressures, as shown below.

TABLE V.

		<i>Average Diameters of palisade Cells and Vein Islets in <math>\mu</math>.</i>	
<i>Vine</i>	{ Reduced pressure	10.0	216
	{ Increased pressure	9.6	152
<i>Sycamore</i>	{ Reduced pressure	11.4	254
	{ Increased pressure	9.2	180

The figures in the above table are averages of fifty measurements. The leaves were *originally* of the same size (before treatment), but after treatment those from increased pressures were much the larger. Since the cells are somewhat smaller in the larger leaves, much more cell division must therefore have taken place under increased pressure.

The decreased size of the vein islets indicates that positive pressures may have a very important role in the production of vascular bundles. Increased pressures certainly produce much larger numbers of veins.

Other arguments available support the evidence given above in showing that cell division rather than cell extension is affected by the pressures employed. If negative pressures stop cell extension and not cell division, then on removing the negative pressure and placing the leaves in water (or applying small positive pressures) rapid elongation ought to ensue. We have never been able to detect any such elongation, and it therefore appears probable on these grounds also that it is cell division which is affected by the treatment. Theoretical considerations lead to a similar conclusion. Cell extension is due to the development of increased osmotic pressures by the young cells. These pressures are usually considered to be considerable,

since cell extension can proceed even in relatively concentrated solutions (see McCallum, 8). It appears, therefore, to be very unlikely that the small tensions employed in our experiments, usually less than 30 cm. of mercury, could materially affect the degree of cell extension, especially as these were applied at the cut end of a twig and would therefore probably be much less in the actual growing regions.

Further, it is more likely that the distribution of water is altered chiefly in those components of the leaf which possess little or no osmotic pressure. The cell sap of woody plants has a higher osmotic concentration, and the pressures recorded for this sap lie between ten and twenty atmospheres (Atkins, 2). It is therefore probable that the water content of the vessels and the cell walls is chiefly affected by the alteration in pressure conditions.

We may now pass on to consider the organization of the leaf meristems in terms of the observed facts. The dividing cells in developing leaves of Dicotyledons are usually situated at the margin. By analogy with the other meristematic tissues of Dicotyledons we may probably assume them to be not or slightly vacuolated and possessing little affinity for water (Pearsall and Priestley, 9). The effect of subjecting such a system to negative pressures would be to tend to dry it out. It is significant in this connexion that the meristematic zones of Vine leaves often become dried and withered after treatment with negative pressures of more than half an atmosphere. A similar marginal drying is often observed in cultural plants in climates where rapid transpiration may occur. Now while such a reduction of water content, if temporary, might lead conceivably to more rapid protoplasmic synthesis, as suggested by Pearsall and Priestley, it would, if long continued, prevent raw materials from reaching the meristematic cells, since these raw materials must diffuse through water. Hence cell division would presumably cease through lack of nutrient material, if the meristematic tissues were partly dried by being subjected to negative pressures for a considerable period. Further, it is not easy to see how the meristems can be irrigated with aqueous solutions, unless these solutions are forced in under pressure. Meristematic tissues are normally extremely impermeable. Not only so, but they are normally separated from the source of aqueous solutions by a layer of elongating cells which is rapidly absorbing water. These cells must tend to dry the adjacent tissues, and particularly the non-osmotic cell walls. But in whatever manner the nutrient solutions reach the dividing cells, it is clear that to do so they must pass through the cell walls. Diffusion through the cell walls would be most rapid if these were saturated with water under pressure; it would slow down and cease as the water content of the walls decreased. These considerations suggest that positive hydrostatic pressures are necessary in order that the meristematic tissues may be irrigated with water, and hence that dissolved materials may reach the dividing cells.

It may be objected that a certain amount of growth took place under negative pressures in our experiments. This was certainly because the negative pressures used were very small. These were applied at the end of a cut shoot and they would tend to be reduced by the shoot. If cut shoots were put in water (e.g. Horse Chestnut, Sycamore) they might show marked exudation from the leaf-tips when these were cut off. Such exudation pressures might be set up not only by the shoots, but also, as shown by Weiler (12), in the leaves themselves, and these pressures would clearly tend to counteract tensions applied at the base of a shoot. We have, in fact, observed small exudations from cut leaf-tips of Horse Chestnut while a negative pressure of 20 cm. of mercury was being applied at the base of the twig. The effect at the meristems of the negative pressures used in our experiments was, therefore, to increase existing tensions or to reduce existing pressures.

The result of these discussions is to suggest that hydrostatic pressures in the plant may directly affect the rate of growth of the meristematic cells, chiefly, it is assumed, through the effect of the pressure on the rate at which nutrient materials enter the meristems.

#### HYDROSTATIC PRESSURES AND LEAF FORM.

When, at a previous stage, the development of typical palmate leaves was considered, it was pointed out that both Vine and Sycamore leaves showed a change in their proportions during their development, the basal lobes becoming relatively larger as the leaves became older. If, therefore, developing leaves are allowed to continue their development, subjected to small positive or small negative pressures, the final cessation of growth ought to be accompanied not only by differences in the sizes of the leaves, but also by differences in their proportions. The leaves with retarded growth, that is, those growing against negative pressures, should show relatively smaller basal lobes. The following tables illustrate the results obtained, and in these and subsequent tables the measurements given are as follows:  $M$ , the main vein; first and second lateral lobes,  $L_1$  and  $L_2$  or  $R_1$  and  $R_2$ , depending on whether they are to the left or right of the main vein;  $C$ , the distance from the junction of petiole and lamina to the incision between the main vein and the first lateral. The measurements are to the nearest half-millimetre; the proportions are given as percentages of the main vein and to the nearest 0.5 per cent. In all cases, the experiments were carried out with twigs of equal size under the pressure conditions named. The leaves on these twigs were roughly similar in size and proportions, and free from abnormalities. Similar leaves and twigs were usually kept in water during the experiments. Their final proportions were intermediate between those of the experimental leaves, but usually resembled more closely the leaves to which positive pressures were applied.



TABLE VI.

*Proportions of Vine and Sycamore after Different Internal Pressure Treatment.*

Pressures.	Vine.		Sycamore.	
	Increased.	Reduced.	Increased.	Reduced.
<i>M</i>	100 (58)	100 (40)	100 (87)	100 (67)
<i>L</i> <sub>1</sub> and <i>R</i> <sub>1</sub>	82, 83	73, 76	93, 95	90, 91
<i>L</i> <sub>2</sub> and <i>R</i> <sub>2</sub>	60, 59	47.5, 50	69, 71	60, 60
<i>C</i>	62	47.5	48.5	43.5

Figures in brackets are lengths in mm.

It is apparent from this table that the supposition with which this section began is correct, namely, that increased pressures lead to a relatively greater development of the basal lobes. Since also the distance *C* (from the centre of the leaf to the upper incision) is relatively increased, the lobes of the leaves tend to be broader when positive pressures are applied. There is, however, another question which requires consideration. Is this relative increase due to uniform growth all round the leaf, or is it due to retarded growth in the basal lobes of leaves treated with reduced pressures? This question involves the consideration of the following table:

TABLE VII.

*Growth of the Lobes of Sycamore Leaves as Percentages of their Original Length.*

Reduced Pressures.			Lobe.	Increased Pressures.		
Original Length.	Increase in Length. mm.	per cent.		Original Length.	Increase in Length. mm.	per cent.
37	3	8	<i>M</i>	40	7	17.5
28	2	7.1	<i>L</i> <sub>1</sub> and <i>R</i> <sub>1</sub>	31	6	19.3
19	1.5	8	<i>L</i> <sub>2</sub> and <i>R</i> <sub>2</sub>	21	5.5	23.3
16	0	0	<i>C</i>	21	3.5	16.0

(Pressures —15–25 cm. of mercury.)

Similar figures result from experiments with Vine. Under positive pressure treatment, the different lobes of the leaf tend to grow at approximately similar rates. With negative pressures, on the other hand, the growth of the basal lobes falls to about half that of the terminal lobe. As the percentages show, the net result is that the original proportions of the leaf remain unaltered after negative pressure treatment, except that apparently no growth takes place at the incision. The latter fact is of interest since it suggests that nutrient solutions do not move very far from the main veins in the lateral direction when negative pressures are applied. The examples taken illustrate the fact that positive pressures tend to produce the broadly lobed type of leaf with prominent basal lobes, while negative pressures give rise to apparently more dissected leaves with small basal lobes.

These facts cannot be adequately discussed without a further consideration of the movement of aqueous solutions in the leaf.

### *Distribution of Water in the Leaf.*

If the pressure treatments used affect the growth of the leaves by altering the supply of water-borne materials to the meristems, there should be

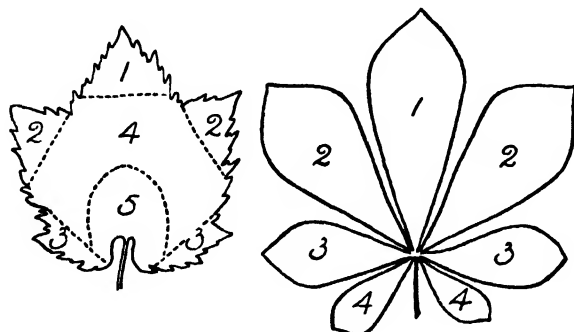


FIG. 2. Method of numbering leaf segments in *Vitis* and *Aesculus*.

detectable differences in the water contents of the leaf meristems after treatment. Vine leaves were cut up as shown in Fig. 2, the cut segments being immediately placed in weighed stoppered bottles and weighed. After drying to constant weight, the percentage of water in each segment was calculated. The results of different estimations are given below, the pressures used being about 30 cm. of mercury.

TABLE VIII.

### *Water Content of Different Parts of Vine Leaves.*

Segment.	Normal untreated leaves (1).		
	Percentage of Water.		
1	72.38	74.87	
2	71.27	76.93	
3	71.92	75.95	
4	72.37	76.17	
5	75.80	76.92	
Leaves after treatment with increased pressures (2).			
1	78.37	74.52	74.00
2	78.01	74.51	76.73
3	78.67	72.42	76.44
4	78.61	73.32	78.94
5	81.18	76.13	78.99
Leaves after treatment with reduced pressures (3).			
1	70.04	70.52	71.80
2	69.23	74.31	67.84
3	69.59	72.42	66.72
4	71.00	71.32	71.47
5	73.94	75.13	74.01

These results are possibly not of a very high order of accuracy, since a certain amount of evaporation from the cut surface may have taken place during the cutting of the leaf. The centre of the leaf (5) always contains most water, however, and if we take segments 1, 2, and 3 roughly to represent the meristems, then we can make the following comparison between the leaves under the different conditions:

*Water Content of Leaf Margins.*

Untreated leaves	71 to 77 per cent.	average	73.9 $\pm$ 0.62
Increased pressures	72 to 79 " "	"	75.95 $\pm$ 0.49
Reduced pressures	67 to 74 " "	"	70.25 $\pm$ 0.52

With reduced pressures there is, therefore, a definite reduction of the water content of the leaf margins, this being associated with reduced growth.

These observations were repeated with Horse Chestnut leaves, as in these leaves evaporation from cut surfaces can be reduced to a minimum. The leaves used were mature or nearly mature and gathered under various conditions in June and July. It will be noted that the water contents vary considerably. The segments are numbered as in Fig. 2. The results are arranged to show the water content of the terminal leaflet (1) and the differences from this figure of the water content of the other leaflets. This brings out the significant point and saves duplicating the table.

TABLE IX.

*Water Content of Leaflets of Aesculus Hippocastanum.*

Segment.	Percentage Water Content.				Average Difference.
<i>Untreated Leaves.</i>					
1	72.15	68.98	68.09	75.20	
2	+ 0.15	+ 0.76	- 0.93	+ 0.31	+ 0.08
3	- 0.57	+ 0.69	- 1.01	+ 0.41	- 0.12
4	- 1.67	+ 0.06	- 1.86	- 0.46	- 0.98
<i>Increased Pressures.</i>					
1	75.62	71.58	74.44	76.45	
2	- 0.11	+ 0.45	- 0.13	- 0.12	+ 0.02
3	- 0.46	+ 1.19	- 0.19	- 0.08	- 0.13
4	- 2.47	- 0.38	+ 0.03	- 1.50	- 1.08
<i>Reduced Pressures.</i>					
1	65.18	73.01	72.49		
2	- 0.37	- 0.14	- 1.29		- 0.60
3	- 0.37	- 1.94	- 1.76		- 1.36
4	- 2.47	- 3.10	- 2.35		- 2.64

In this table differences of less than 0.5 per cent. are probably not significant. To err on the safe side we may disregard differences of less than about 1.0 per cent. We can then conclude that leaves treated with negative pressures show a decided reduction in the relative water content

in their basal segments, and there is always a tendency towards lower water content in the third segments. The untreated leaves and those treated with increased pressures are obviously alike and may be considered together. As a whole the water content of the various leaflets tends to resemble that of the terminal leaflet. In four out of the eight determinations for the basal leaflets, however, the water content is significantly lower than that of the terminal leaflets. We may conclude, therefore, that there is a decided tendency for these basal leaflets to have a lower water content. The average differences, although hardly justified by the small number of determinations, serve to bring out the conclusions at which we have arrived.

In these results, one point seems worthy of further investigation—the fact that either in leaves untreated or treated with positive pressures, the lowest lobes tend to contain least water. It seems possible that this might be due in some measure to the large angle which these leaflets make with the petiole and main vein, and this might to some extent prevent the flow of water into these leaflets. To examine the question in more detail, the lamina was stripped off a Horse Chestnut leaf and only three centimetres of vein left for each leaflet. The veins were inserted into narrow glass tubes of equal bore sealed at one end. Water was then forced up the petiole under pressure. Drops exuded from the cut veins, and these were collected in the tubes and subsequently measured.

TABLE X.

*Delivery of Water through Main Veins.*

<i>Vein</i>	<i>M.</i>	<i>R</i> <sub>1</sub> .	<i>L</i> <sub>1</sub> .	<i>R</i> <sub>2</sub> .	<i>L</i> <sub>2</sub> .	<i>R</i> <sub>3</sub> .	<i>L</i> <sub>3</sub> .	<i>Pressure.</i>
Angle with main vein	0	45°	45°	90°	90°	135°	135°	
1. Length of vein in mm.	225	215	228	167	172	110	122	60 cm. of mercury for 2 hours.
Height of water in mm.	43	42	45	24	25	8	9	
Area of leaflet in sq. cm.	98.1	92.8	100	65.2	67.7	34	39.9	
2. Length of vein in mm.	163	152	151	108	125	80	95	30 cm. of mercury for 20 hours.
Height of water in mm.	17	12	15	5	7	0	1	
Area of leaflet in sq. cm.	66	54.6	58.7	35.5	2	14.5	21.6	

These examples were chosen because the angles the veins make with the main vein petiole line were very regular. There is clearly a direct relation between the length or area of the leaflet and the amount of water which can be forced into it under pressure. This is illustrated graphically in Fig. 3. On the other hand, no water is delivered to leaves of less than about 90 mm. in length. In this respect all our results are in agreement. Three factors must be considered in explaining this fact. In the first place,

the leaflets during the unfolding phase differ in their proportions from older leaves and are oriented at quite different angles to the petiole. The results of our experiments apply only to the later stages, and our starting-point is therefore the average length of the leaflets at the time when they assume the mature position. We estimate this starting-point (see Stage 2, Table II) at between 100 and 75 mm. Deducting this previous growth as given

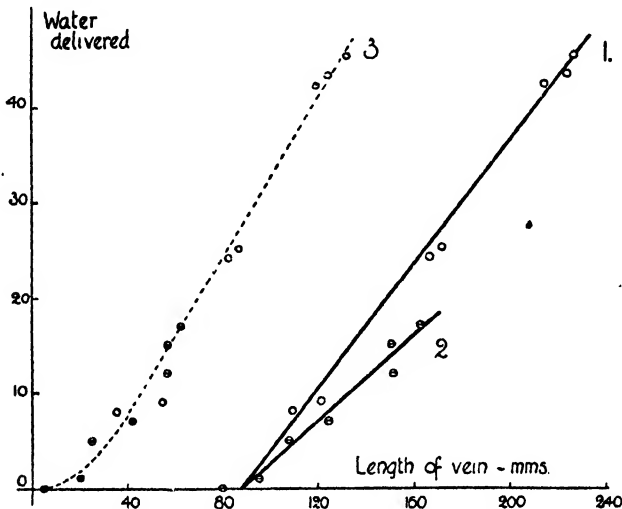


FIG. 3. Relation between amount of water delivered through a given vein (in arbitrary units) and the length of the vein. 1 and 2, Series 1 and 2, Table X. 3, see text.

in Table II, we get very approximately the amounts of growth which take place with the veins in their final positions, and, as shown in Fig. 3, the quantity of water delivered in the experiments still remains approximately proportional to the growth in the later stages. A closer approximation might possibly be obtained if all the details for a single leaf were available.

The second possibility to be considered is that the xylem elements become partly blocked up in the smaller leaflets, so that the veins are relatively less efficient. The leaf used for the second set of figures in Table X was examined for evidence on this point. The veins were sectioned and the area of xylem carefully estimated in each. The average diameter of the xylem vessels was also determined, and from these figures the average numbers of vessels in the conducting strands were calculated. The volume  $V$  of liquid delivered in time  $t$  through a tube of length  $l$  and radius  $r$  is given by the equation

$$V = \frac{\pi p r^4 t}{8 l v},$$

where  $p$  is the pressure difference between the ends of the tube, and  $v$  is the coefficient of viscosity of the liquid. For our data  $p$ ,  $t$ ,  $l$ , and  $v$  are the same

for the various veins, so that the relative delivery *R.D.* of the various veins will be given approximately by

$$R.D. = \text{number of vessels} \times (\text{average radius of vessels})^4.$$

The data obtained are given in the following :

TABLE XI.

<i>V</i> Vein.	Relative No. of Vessels.	Diameter of Vessels. μ.	Relative Flow per Vessel.	Relative Delivery.	Compara- tive ( <i>R.D.</i> ).	Observed Delivery. Comparative ( <i>O.D.</i> ). mm.
M	452	28	1	452	1	17
K <sub>1</sub>	450	25.6	0.7	315	0.7	12
L <sub>1</sub>	350	28	1.0	350	0.78	15
R <sub>2</sub>	266	24.0	0.54	144	0.32	5
L <sub>2</sub>	347	25.2	0.66	232	0.51	7
R <sub>3</sub>	295	17.2	0.14	41	0.09	0
L <sub>3</sub>	265	22	0.38	100	0.22	1

Two comparative columns in this table give the relative and actual deliveries in proportion to those of the main vein. While there is fair agreement between the figures for calculated relative delivery and the actual delivery, it is noticeable that for all the smaller veins the actual delivery is less than the calculated figure. Any general error in the method of estimating the area of the xylem would not produce this difference, since it would (and does) affect all the figures for relative delivery proportionately. The difference is of course particularly significant in the case of the basal lobes, since these deliver little or no water although they have well-developed xylem. There is no anatomical evidence suggesting that some of the veins are partly blocked, and, indeed, water can be forced through if sufficient pressures are used or the whole vein detached and treated separately. Further, the difficulty of getting water through the lower veins does not show any material increase with age. Leaves taken in June give similar results to those taken in late September. It is necessary therefore to consider the third factor, namely, the angle at which the veins come off the petiole. If the figures in Table XI are arranged to show the relation between  $\frac{\text{observed delivery (O.D.)}}{\text{calculated delivery (R.D.)}}$  and the angle between the vein and the mid-vein, we get

Angle	0°	45°	90°	135°
$\frac{O.D.}{R.D.}$	1	1.1	0.94, 0.80	0.027

Thus the efficiency of the conducting channels seems to fall off very markedly if they make an angle of over 90° with the main vein, when the petiole and main vein lie in the same straight line.

It is probable that in our experiments the flow of water along the main vein tends, if it is sufficiently rapid, to cause a suction, or at any rate to neutralize the positive pressures, in the veins making a large angle with the direction of flow. Hence no water is exuded from these veins. It is clear

that water flowing rapidly past a pipe oriented at such an angle would draw water out of the pipe by setting up a 'back pressure', a familiar fact to water engineers. The degree to which such a back pressure could be developed would be largely dependent upon the rate at which the water was flowing. Could it occur in normal leaves? Assuming that the existence of the back pressure is shown under the conditions of our experiments, then we can apply the second series of results to the consideration of the problem. In this experiment the results were obtained after twenty hours, using a pressure of 30 cm. of mercury. For the largest vein the actual flow of water during this period was 0.425 gm. and the rate was therefore 21.25 mg. per hour. This leaflet had an area of 66 sq. cm. Assuming that it normally transpired at the rate of 0.5 mg. per sq. cm. per hour, which is a very low estimate, chosen to represent a minimal rate, then the total flow of water into the leaflet to replace that lost by transpiration would be 33 mg. per hour, half as much again as that flowing in our experiment. It is clear that if the falling off in water delivery to the basal veins in our experiments is due to the back-pressure effect, then a similar effect must occur in the leaves owing to transpiration currents. This would satisfactorily account for the lower water content which is normally found in the basal lobes, and also for the stoppage of growth in these lobes when they assume their final orientation. The stoppage of growth could, under these circumstances, be attributed to the local reduction of hydrostatic pressure. One important general conclusion remains to be drawn from these results. Any factor which causes increased transpiration will also cause an increased rate of flow into the leaf, and hence an increase in the back-pressure effect in the basal lobes. Increased transpiration therefore means reduced hydrostatic pressures in the basal lobes, and hence reduced rates of growth in these lobes, even if we assume that the hydrostatic pressure at the end of the petiole remains constant. This conclusion is illustrated by some experiments to be described later, and also by the work of Eberhardt (4). The latter grew plants in moist air and in dry air, and although he gives no dimensions of the leaves under these conditions, his figures enable a comparison to be made. Two cases are of interest as dealing with lobed leaves. In *Spiraea Lindleyana* the effect of dry air, i. e. increased transpiration, is to cause a general reduction in leaf size, but no proportionate decrease in the lateral lobes. Since in this species the veins of the lateral lobes make an angle of 40 to 45° with the main vein, no back-pressure effect would be expected, and hence no proportionate reduction in the size of the lateral lobes. In *Ricinus communis*, however, where the veins of the lower lobes make angles of 90° or more with the main vein, there is in dry air a decided reduction in the size of the basal lobes, which may reasonably be attributed to the back-pressure effect.

It is of interest to consider, in terms of pressure effects, the growth of

Vine and Sycamore leaves. When these are young they probably behave like the mature Horse Chestnut leaf, since the orientation is similar. On the other hand, in the mature stage these leaves are oriented so that the lamina is approximately at right angles to the petiole, and hence all the veins make approximately the same angle with the petiole. Under these circumstances there can be no back-pressure effects, and the pressures at the ends of the veins should approach the same value and the leaves ought to tend to grow more or less uniformly all round. This they do under normal conditions and under positive pressures. Under negative pressures, however, growth is proportional to the length of the lobe. This seems to suggest that the pressures at the ends of the basal lobes are less than those at the end of the terminal lobe. It may be that the power of neutralizing tensions is in some degree proportional to the size of the leaflet. If this were so, under these pressure conditions we should expect growth to be approximately proportional to the size of the leaflet. This is actually the result obtained with the lobes of Vine and Sycamore leaves treated with reduced pressures.

Greatest breadth as percentage of length of the main vein in leaves grown in dry and moist air (Eberhardt).		
	<i>Spiraea Lindleyana.</i>	<i>Ricinus communis.</i>
Dry air	88	40
Moist air	89	64

Finally, it may be pointed out that the results of the experiments in which leaves were clipped in various positions can be readily explained on the basis outlined above. Here again, if the lamina is fixed at right angles to the petiole, the rates of growth in the various lobes tend to approach equality. If the lamina is in the same plane as the petiole, the rates of growth of the basal lobes are relatively greatly reduced, presumably owing to the large angle these lobes make with the petiole and the resulting tendency towards back pressures.

#### *Seasonal Variations in Leaf Form.*

While there are many exceedingly familiar cases of variation in leaf form, the type of variation found in palmate leaves does not appear to have received any detailed description or explanation. This type is very well shown in Sycamore. The leaves first developed in spring are broad, with prominent basal lobes. They are succeeded in summer by subsequent leaves which are much reduced in area, more dissected, and possessing narrower lobes, and have much smaller basal lobes. These leaves may continue to appear until August, and their characteristics become more and more marked the later they develop. The summer conditions under which these later leaves develop are clearly conditions which tend to produce negative pressures in the tree, namely, a higher rate of transpiration, a



longer duration of light, and often reduced water absorption from the soil. Further, the data as to the seasonal march of pressures in trees (Pfeffer, 10) indicate that the lowest pressures occur during this summer period. The characteristics of the late summer leaves are also exactly those of leaves allowed to develop under experimental conditions of negative pressure. It may therefore be concluded with some certainty that one of the main factors in seasonal leaf variation is the variation of the internal hydrostatic pressures.

A number of other plants show similar variations to the Sycamore, namely *Aesculus Hippocastanum*, *Acer campestre*, *Althaea*, and *Platanus orientalis*. In the undivided leaves, *Populus nigra* and *Tilia europaea*, the summer leaves tend to become smaller and proportionately narrower. Eberhardt's results (4) show that this type of leaf is produced in dry air by *Populus nigra*, so that probably we are also dealing with a reduced pressure effect in this case also. A point of interest is that all the above species of trees except *Acer campestre*, which was not examined, show exudation pressures during the growth of the spring leaves. The smaller types of leaves with no marked exudation have not been observed to show marked seasonal variation.

A factor in seasonal variation which as yet remains unestimated is the possible alteration in the type or proportions of the various food substances reaching the plant meristem.

#### SUMMARY.

1. The development of some typical palmate leaves is considered in relation to water-supply and internal hydrostatic pressures.

2. Evidence is presented showing that negative hydrostatic pressures cause leaf growth to stop or to slow down, and favouring the assumption that rapid growth is usually associated with the presence of positive hydrostatic pressures.

3. It is shown that after treatment with negative or reduced pressures palmate leaves become smaller, more dissected, and have reduced basal lobes when compared with similar leaves after positive pressure treatment.

4. There is evidence to show that when the angle between a lateral vein and the main vein of a palmate leaf approaches or exceeds  $90^\circ$ , the supply of water to the lateral vein becomes reduced. A possible explanation of this fact is discussed.

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# The Bisexuality of Individual Strains of *Coprinus Rostrupianus*.

BY

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With Plate VI and seventeen Figures in the Text.

## I. INTRODUCTION.

IN 1918 Mlle Bensaude, employing experimental and cytological methods, proved conclusively that the phenomenon of heterothallism occurs in the Hymenomycetes (1). She isolated two monosporous mycelia of *Coprinus fimetarius* and found that they remained in the haploid (primary) condition during eight months of continuous cultivation, without any sign of fruiting, but that when they were paired they united and produced a diploid (secondary) clamp-bearing mycelium which fruited readily. In 1919 Kniep showed by experiment that *Schizophyllum commune* is heterothallic, and stated that many other Hymenomycetes also exhibit this sexual condition (11). Further discoveries of heterothallism in Hymenomycetes have been made: by Kniep, working with *Aleurodiscus polygonius* (12); by Miss Mounce, working with *Coprinus lagopus* and *C. niveus* (15, 16); by Vandendries, working with *Collybia velutipes*, *Hypholoma fasciculare*, *Panaeolus campanulatus*, *P. separatus* (= *Anellaria separata*), *P. fimicola*, and *Coprinus radians* (18, 19, 20); and by Brunswik working with sixteen species of the genus *Coprinus* (3).

In papers published in 1921 and 1922, Miss Mounce first definitely established that the phenomenon of homothallism occurs in the Hymenomycetes (15, 16). She made monosporous cultures of *Coprinus sterquilinus* and of *C. stercorearius* and found that the mycelia soon developed clamp-connexions and subsequently produced perfect fruit-bodies. She succeeded in growing *C. sterquilinus* in pure monosporous cultures for seven successive generations. In unpublished work, reported by Buller (5), she also found that *C. narcoticus* is homothallic. Confirmation of homothallism, as found

by Miss Mounce, has been obtained in the Winnipeg laboratory: for *C. sterquilinus* by Hanna and the writer, working independently; and for *C. stercorarius* by the writer.<sup>1</sup> The species of *Coprinus* determined by Brunswick as being homothallic are: *C. sterquilinus*, *C. stercorarius*, *C. narcoticus*, and *C. ephemeroideus* (3).

Individual sexual strains of *Schizophyllum commune* and *Aleurodiscus polygonius* have each been shown by Kniep to possess four sexually different kinds of spores (12, 13). In order to explain his results, Kniep assumed that in these strains sex is determined by two allelomorphic pairs of factors which are present in the fusion nucleus of the basidium and which, during the two subsequent nuclear divisions, become segregated in accordance with Mendelian principles. If these pairs of factors are represented by the symbols (Aa) and (Bb), then the fusion nucleus of the basidium must have the genetic constitution (AaBb). During reduction and division of the fusion nucleus, the sex factors become segregated, with the result that, finally, there are formed four sexually different kinds of spores: (AB), (ab), (Ab), and (aB). Only those spores without a common factor unite sexually in the mycelial stage. Thus, monosporous mycelia bearing the factors (AB) and (ab), when paired, unite sexually to form a diploid clamp-bearing mycelium; whereas monosporous mycelia bearing the factors (AB) and (aB) are not able to do this because (B) is a common factor and two like sex factors repel one another. Similarly, while mycelia bearing the factors (Ab) and (aB) can unite sexually, mycelia bearing the factors (AB) and (Ab) cannot.

Kniep observed that, under certain abnormal culture conditions, each basidium of *Aleurodiscus polygonius* shoots away its four spores in a single mass (12). He isolated thirty-five of these spore-masses, and shook the four spores of each mass apart in a flask containing nutrient agar. Subsequently, the four mycelia produced from the four spores were removed from the flask, grown separately, and then mated in all possible ways. As a result, Kniep found that each basidium of *Aleurodiscus polygonius* bears two pairs of spores, one pair of one sex, and the other pair of another and opposite sex. He also found that the basidia of a single sexual strain were of two kinds, one kind bearing spores (AB), (AB), (ab), and (ab), and the other bearing spores (Ab), (Ab), (aB), and (aB). Kniep concluded from these results that the reduction of the chromosomes takes place in *A. polygonius* in the *first* division of the fusion nucleus, and not in the second.<sup>2</sup>

Hanna (8), working with *Coprinus lagopus*, found that the spores from any individual fruit-body belong to four sexually different groups, and thus

<sup>1</sup> This confirmation has not previously been announced.

<sup>2</sup> Since this paragraph was written Professor Kniep has informed Professor Buller *in litt.* that, since the publication of his paper, he has found a few spore-masses which included spores of all four sexes: (AB), (ab), (Ab), (aB).

resemble those found by Kniep in *Schizophyllum commune* and *Aleurodiscus polygonius*. However, Hanna's basidial analyses show that, while some basidia bear spores of two sexes only, a pair of one sex and a pair of another and opposite sex, other basidia bear spores of all four sexes: (AB), (ab), (Ab), and (aB). The occurrence of four sexually different kinds of spores on a single basidium in *Coprinus lagopus* is regarded by Hanna as proving that the reduction process in the basidium in this species takes place in the second division of the nucleus, and not in the first.

Funke (6) isolated the four spores of individual basidia of *Hypholoma fasciculare*, *H. capnoides*, and *Collybia velutipes* and determined their sex, with results similar to those obtained by Hanna. Like Hanna, from the fact that some of the basidia had produced spores of four different kinds, he concluded that reduction takes place during the second division of the basidial nucleus and not during the first.

In view of the fact that homothallic and heterothallic quadrisexual species had already been found in *Coprinus*, it seemed not unlikely that this genus might also include heterothallic bisexual species. Such a species the writer has had the good fortune to find in *Coprinus Rostrupianus*. While the investigations here recorded were in progress, Vandendries (20) and Brunswik (3) announced that they also had discovered bisexual species of *Coprinus*.

Experiments made by Kniep, Vandendries, Brunswik, Hanna, and the writer indicate that every species of Hymenomycetes is made up of many different sexual strains which are perfectly fertile *inter se* (*vide infra*). Thus Hanna (8) found six interfertile geographical strains of *Coprinus lagopus*. While each of these strains was quadrisexual, the six strains collectively represented twenty-four different sexes. In this paper, when a species is spoken of as *bisexual* or *quadrisexual*, it must be understood that these terms have reference to the sexual condition of each of the individual strains of which the species is composed and not to all the strains taken collectively. When all the sexual strains are considered together, each species of *Coprinus*, &c., must be regarded as *multisexual*.

In his most recent paper Vandendries (21) records that a large percentage of the monosporous mycelia of his bisexual species, *Coprinus radians*, in the course of six months, changed spontaneously from the haploid to the diploid condition, in consequence of which he now regards *C. radians* not as heterothallic but as *hetero-homothallic*. As will be seen from a series of observations given at the end of this paper, *C. Rostrupianus* behaves in exactly the same manner as *C. radians*; for, in the course of some months, a large percentage of the haploid mycelia change spontaneously into diploid mycelia. However, since actual tests made at intervals showed that each of twenty-six monosporous mycelia of *C. Rostrupianus* retained its unisexual character for the first nine weeks of its

existence, the individual strains of the fungus have been regarded as strictly bisexual during this period.

*Coprinus Rostrupianus* is a large-spored sclerotium-producing species. On November 14, 1923, in a meadow at the Manitoba Agricultural College, Winnipeg, a number of large irregular blackish sclerotia (cf. Pl. VI, Figs. 1 and 2) were found by Professor A. H. R. Buller embedded in an old cow-dung plat. They were brought to the laboratory, where, in a moist chamber, in the course of about two weeks, they began to produce fruit-bodies (Pl. VI, Figs. 3 and 4). Each fruit-body took about ten days to develop to maturity. The scales on the pileus were white and made up of slender septate hyphae, the individual cells of which were often branched. The cystidia were cylindrical and very long—up to 0.3 mm. in length—and they could be seen with the naked eye in expanding pilei bridging adjacent gills as described by Buller for *C. atramentarius* (5). The spores were densely black, spade-like in general appearance, having three dimensions (Text-fig. 1, *a*, *b*, *c*), the length in dry spore-deposits (Pl. VI, Fig. 5) being 14–16  $\mu$  and the breadth 11.5–13  $\mu$ , and provided with an unusually prominent black apiculus. In October, 1924, Professor Buller found the fungus again, once more in a meadow at the Manitoba Agricultural College and a second time at Kenora, Ontario, about 100 miles east of Winnipeg.

*Coprinus Rostrupianus* was first described in 1897 by E. Chr. Hansen, who found it in Denmark coming up on cow-dung (9). The fruit-bodies always originated from a sclerotium, and the sclerotia were larger and more irregular than those of *C. stercorarius*. Hansen illustrated his paper with sketches of the spores, the pilear covering, &c.; but, unfortunately, he did not give a drawing of the whole fruit-body. However, from his description, there was no difficulty in identifying the fungus found at Winnipeg as *Coprinus Rostrupianus*. Lange (14), in his monograph of the genus *Coprinus* (1915), includes *C. Rostrupianus*; but, as he could not find any sclerotia attached to the fruit-bodies, his identification seems doubtful.<sup>1</sup> *C. Rostrupianus* is not mentioned by Ricken (17) in 'Die Blätterpilze' (1915), and hitherto it has not been recorded for North America. Hence we may conclude that *C. Rostrupianus* is a somewhat rare fungus or, at least, one that is easily overlooked by mycologists.

A preliminary announcement of some of the chief results of this paper has been made by Buller (4).

## II. METHODS.

Spore-deposits from each fruit-body were collected separately on dry sterilized glass slides (Pl. VI, Fig. 5). The slides bearing spore-deposits

<sup>1</sup> In laboratory cultures, without exception, it was observed that fruit-bodies of *C. Rostrupianus* never developed directly on ordinary mycelium, but always from sclerotia.

taken from a single fruit-body were wrapped together in white writing-paper and labelled with the number of the fruit-body and the date on which the spores were shed. Thus the wrapped slides were stored free from danger of contamination by spores from other fruit-bodies.

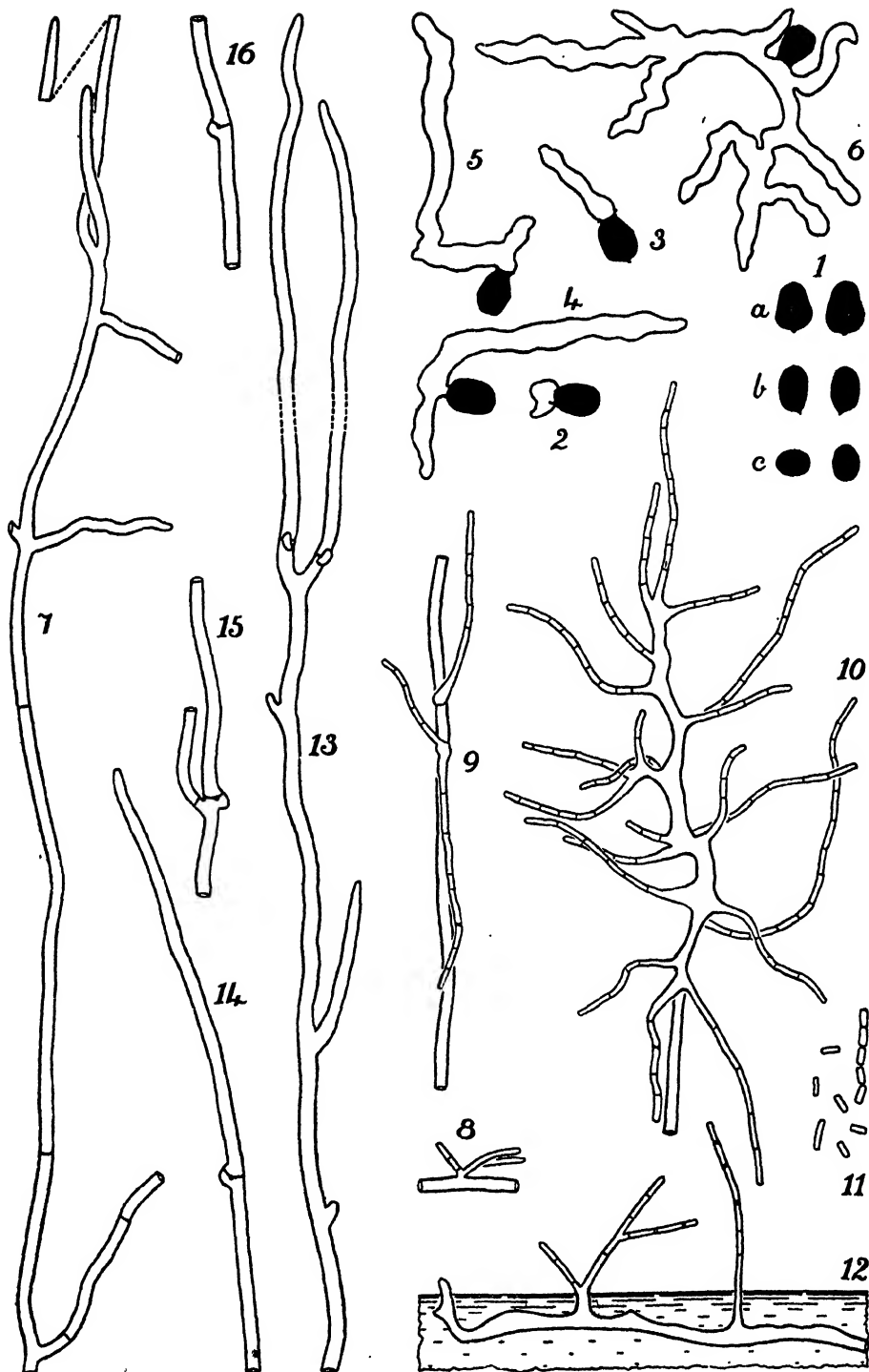
The spores were isolated and sown by the dry-needle method (7), and dung-agar was used as the medium for germination. In preparation for removing a new spore from a dry spore-deposit, the sewing needle was never heated in a flame, but was always run through clean linen. The surface of the slide or cover-glass which receives the spore-deposit should be flamed to remove any organic film which, if present, may make it difficult or even impossible to pick up the spores. These details were not mentioned by Hanna (7).

The germination of fresh spores taken from deposits made in the spring of 1923 varied from 60 to 80 per cent., while that of spores taken from deposits made in November, 1924, and tested in lots of fifteen to twenty spores, showed 100 per cent. germination. After spores had been stored for a month or longer, their power of germination was found to have considerably decreased. Some germinating spores are shown in Text-figs. 2-6.

The four spores of single basidia were obtained for isolation by what may be called the *coverglass-contact method* (4, 8), which will now be described. A fruit-body about to undergo autodigestion is removed from the culture-dish in which it is growing, and one of its gills is cut away and laid flat on a glass slide. A cover-glass, held with forceps, is then lowered until it touches the hymenium lightly, whereupon it is raised, inverted, and examined under the microscope. In certain places on the cover-glass the spores can be seen adhering by their apices in groups of four (Pl. VI, Fig. 6). The spatial arrangement of each tetrad is exactly like that of the four spores on a basidium when the hymenium is examined microscopically from above, so that there can be no doubt that each tetrad has actually been derived from the four spores of a single basidium. By means of the dry-needle method (7) the spores surrounding a well-defined spore-tetrad are first removed, and then the four spores are picked up one by one and sown separately in the culture medium.

The dung-agar used as the culture medium was prepared as follows: About 300 grm. of fresh horse-dung were stirred up with 1,000 c.c. water. The mixture was boiled for fifteen minutes, and then filtered twice through cotton-wool. Water equivalent to that lost through boiling and filtering was then added, agar at the rate of 1.2 per cent. was stirred in, and the whole boiled again until all the agar had melted. The medium was then filtered through cotton-wool, tubed, and sterilized for one hour at fifteen pounds pressure.





TEXT-FIGS. 1-16, *Coprinus Rostrupianus*.  $\times 470$ .

### III. CRITERIA OF SEX.

Hans Kniep (10) and Mlle Bensaude (1), working independently, discovered that in a *haploid* mycelium in which the nuclei occur singly and separated from one another, often one in each cell, the septa are always simple and never accompanied by clamp-connexions; but that in a *diploid* mycelium in which the nuclei occur in pairs and divide conjugately clamp-connexions are present. Thus the presence of clamp-connexions is an outward and visible sign that the mycelium of which they form a part is in the diploid and not in the haploid condition. This criterion of sex, i. e. the presence or absence of clamp-connexions, was the chief one employed in determining the nature of the sexual reaction when two mycelia of *Coprinus Rostrupianus* were mated. A haploid mycelium of *C. Rostrupianus* with simple septa is shown in Text-fig. 7, and a diploid mycelium with the septa accompanied by clamp-connexions in Text-figs. 13–16.

A haploid mycelium of *Coprinus Rostrupianus* bears numerous oidia at the surface of the culture medium (Text-figs. 8–12), whereas a diploid mycelium never bears any. Hence, macroscopically, a haploid mycelium has a somewhat floury appearance, whereas a diploid has not. Moreover, a haploid mycelium sends out its lateral branches at a greater angle than

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TEXT-FIGS. 1–16. *Coprinus Rostrupianus*. × 470.

TEXT-FIG. 1. Ripe spores viewed: *a*, from in front or from behind; *b*, from the side; and *c*, from the top.

TEXT-FIG. 2. A spore germinating in dung-extract: an irregular vesicle has grown out from the germ-pore.

TEXT-FIGS. 3, 4, and 5. Spores germinating in dung-extract, showing the production of germ-tubes from the vesicles at the germ-pores.

TEXT-FIG. 6. A spore, germinating in dung-extract, which has produced a haploid or primary mycelium which is as yet unicellular.

TEXT-FIG. 7. A branch of a haploid mycelium developed in dung-agar, showing simple septa and lateral hyphae growing outwards from the leading hypha at various angles.

TEXT-FIGS. 8–12. These show the production of aerial chains of oidia on haploid hyphae submerged in dung-agar.

TEXT-FIG. 8. A haploid hypha with an oidiophore bearing three hyphae which are developing into chains of oidia.

TEXT-FIG. 9. A haploid hypha with two oidiophores, one bearing a single chain of oidia, the other bearing two chains.

TEXT-FIG. 10. An irregularly thickened terminal haploid hypha bearing numerous simple or branched oidiophores, each continued into a chain of oidia.

TEXT-FIG. 11. Several isolated oidia and one chain of oidia which became separated from the parent mycelium on the addition of water.

TEXT-FIG. 12. A diagram of a vertical section through dung-agar showing a submerged haploid hypha with three oidiophores which have grown towards the surface of the medium. The basal oidiophore bears a single chain of aerial oidia and the central one two chains of oidia (one branched), while the apical one is about to produce oidia.

TEXT-FIGS. 13–16. Hyphae of a diploid or secondary mycelium developed on dung-agar, showing septa with clamp-connexions. The two clamp-connexions in Fig. 13 are seen from above, those in Figs. 14, 15, and 16 from the side. In Fig. 15 there is a plain septum as well as one with a clamp-connexion. The lateral hyphae shown in Figs. 13 and 15 make an acute angle with the parent hyphae. In Fig. 13 the two terminal hyphae, as indicated by the broken lines, have been slightly shortened.

a diploid mycelium (cf. Text-figs. 7 and 13). Thus the presence or absence of oidia and the nature of the branching form two further criteria for distinguishing between haploid and diploid mycelia ; but, in the present investigation, the criterion finally relied upon for determining sex was always the presence or absence of clamp-connexions.

Both haploid mycelia of monosporous origin and diploid mycelia of polysporous origin were grown on horse-dung in pure cultures, and it was found that both haploid and diploid mycelia gave rise to sclerotia (for diploid sclerotia *vide* Pl. VI, Figs. 1 and 2). However, when the sclerotia were set out in damp sand, only those of diploid origin gave rise to perfect fruit-bodies (Pl. VI, Figs. 3 and 4), whereas those of haploid origin either grew out into the sand in the form of a thick mycelium or produced rudimentary fruit-bodies only. It therefore appears that in *Coprinus Rostrupianus* the production of spore-bearing fruit-bodies is limited to those sclerotia which arise from a diploid mycelium. Mlle Bensaude (1) used the criterion of fruiting in the determination of the sexual reactions of *C. fimetarius*, but this criterion could not be conveniently applied in the present investigation, owing to the fact that the mycelium of *C. Rostrupianus* first forms sclerotia, and the sclerotia usually require a period of rest of some weeks or months before they germinate.

#### IV. EXPERIMENTAL RESULTS.

By employing the methods described, the four spores from each of a number of basidia of fruit-body No. 1 were transferred to dung-agar for germination. All four spores of one basidium germinated perfectly, three of another basidium, and two of each of two other basidia. All possible crossings of these eleven mycelia were made on dung-agar plates in the manner described by Hanna for *Coprinus lagopus* (8). The plates were examined six days after the pairings had been made. When two mycelia of like sexual reactions happened to be paired on a plate, the compound mycelium resulting remained in the haploid condition ; whereas, when two mycelia of opposite sex happened to be paired, the compound mycelium resulting became diploid and could easily be distinguished from a haploid mycelium by the presence of clamp-connexions. Before examining the hyphae for clamp-connexions microscopically, the plates were always viewed macroscopically, and then, as a rule, the haploid mycelia could be distinguished by the presence or absence of the floury oidia at the surface of the agar.

In quadrisexual Hymenomycetes, e. g. in *Schizophyllum commune* (13), *Aleurodiscus polygonius* (12), *Coprinus lagopus* (8), *C. niveus* (3, 16), *C. micaceus* and *C. picaceus* (3), investigated by others, as well as in *Collybia velutipes* and *Coprinus curtus* studied by the writer before the present investigation was undertaken, when all possible pairings are made between

ten or more monosporous mycelia of an individual sexual strain, the mycelia fall into *four* sexual groups, the mycelia of any one group giving a positive reaction with only one of the three other groups. When the plates containing all possible crossings of the eleven monosporous mycelia of *Coprinus Rostrupianus* came to be examined, it was found that the mycelia fell into *two* groups only and not into four. It therefore seemed possible that the strains of *C. Rostrupianus* are bisexual and not quadrisexual. Of the four spores of one basidium, two were of one sex and the other two were of another and opposite sex; of the three spores of another basidium, two were of one sex and one of another and opposite sex. The remaining spores were all of one and the same sex. Of the eleven spores which had developed mycelia eight were of one sex and three of another and opposite sex.

It seemed possible that the occurrence of only two sexually different kinds of monosporous mycelia in the experiments just described might have been due to the fact that, while the species was in reality quadrisexual, the eleven mycelia chosen might have belonged by chance to only two of the four possible sexual groups. A new set of experiments was therefore undertaken. Twelve spores derived from six different basidia germinated, and the resulting mycelia were then crossed in all possible ways. The results of the crossings are embodied in Table I. A (—) sign denotes that the two mycelia placed together in the Petri dish remained in the haploid condition and therefore did not give a positive sexual reaction, while a (+) sign denotes that the two mycelia developed a diploid, clamp-bearing mycelium, and therefore reacted positively towards one another.

It will be seen from this table that the twelve mycelia, as in the previous experiment, fall into only two groups, mycelia Nos. 1, 2, 6, 8, 10, 11, and 12 being of one sex, and mycelia Nos. 3, 4, 5, 7, and 9 being of another and opposite sex. The chances that with twelve monosporous mycelia only two sexual groups should be represented out of a possible four seemed very slight, and the evidence therefore pointed to each individual fruit-body of *Coprinus Rostrupianus* as being bisexual. However, as no clear-cut case of bisexuality in the Hymenomycetes had previously been recorded,<sup>1</sup> it seemed advisable to extend the investigation with a view to substituting a certainty for a probability. Therefore, in the autumn of 1924, more extensive experimental work was undertaken.

By means of the coverglass-contact and dry-needle methods already described, all the spores from fourteen different basidia of fruit-bodies Nos. 3 and 4 were sown separately in the culture medium. All the fifty-six spores germinated.

<sup>1</sup> Vandendries (18), in 1923, had reported 'bipolarity' in *Panaeolus separatus* and *P. campanulatus*, but his tables contained so many exceptions, or 'hermaphrodites' as he called them, that his experiments did not prove conclusively that bisexuality occurs in the Hymenomycetes.

TABLE I.

*All Possible Pairings of Twelve Monosporous Mycelia derived from Six Different Basidia.*

		1				2		3		4		5		6	
		1	2	3	4	5	6	7	8	9	10	11	12		
1	1	-	-	+	+	+	-	+	-	+	-	-	-		
	2	-	-	+	+	+	-	+	-	+	-	-	-		
	3	+	+	-	-	-	+	-	+	-	+	+	+		
	4	+	+	-	-	-	+	-	+	-	+	+	+		
2	5	+	+	-	-	-	+	-	+	-	+	+	+		
	6	-	-	+	+	+	-	+	-	+	-	-	-		
3	7	+	+	-	-	-	+	-	+	-	+	+	+		
	8	-	-	+	+	+	-	+	-	+	-	-	-		
4	9	+	+	-	-	-	+	-	+	-	+	+	+		
	10	-	-	+	+	+	-	+	-	+	-	-	-		
5	11	-	-	+	+	+	-	+	-	+	-	-	-		
6	12	-	-	+	+	+	-	+	-	+	-	-	-		

TABLE II.

*All Possible Pairings of Four Monosporous Mycelia derived from the Four Spores of a Single Basidium.*

	1	4	2	3
1	-	-	+	+
4	-	-	+	+
2	+	+	-	-
3	+	+	-	-

The sexual constitution of the four mycelia produced by the four spores of each of the fourteen basidia was first determined. The four mycelia were crossed with one another in all possible ways. It was found that each of the fourteen basidia had produced two pairs of spores, one pair of one sex,

and the other pair of another and opposite sex, as shown in Table II, which is representative of the behaviour of all fourteen sets of spores. The

TABLE III.

*All Possible Pairings of Twenty-eight Monosporous Mycelia derived from Seven Different Basidia of Fruit-body No. 4.*

7				8				9				10				11				12				14					
1	4	2	3	3	4	1	2	1	3	2	4	1	2	3	4	1	3	2	4	1	2	3	4	1	4	2	3		
7	1	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	4	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	2	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	3	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
8	3	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	4	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	1	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	2	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
9	1	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	3	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	2	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	4	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
10	1	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	2	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	3	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	4	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
11	1	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	3	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	2	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	4	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
12	1	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	2	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	3	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	4	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
14	1	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	4	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	2	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	3	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-

numbers 1, 2, 3, and 4 are merely arbitrary numbers used to designate the four mycelia before the pairings were made. As shown in the table, spores Nos. 1 and 4 belong to one sexual group, and spores Nos. 2 and 3 to another and opposite sexual group.

In order to determine whether or not the two sexes represented by the

two pairs of spores of any one basidium are identical with the two sexes represented by the two pairs of spores of any other basidium, all possible crossings between twenty-eight spores derived from seven basidia of fruit-body No. 4 were made. The results are embodied in Table III.

As shown in the table, the four mycelia derived from any one of the seven basidia behaved sexually in exactly the same manner as the four mycelia derived from any of the other six basidia. The results embodied in Table III therefore justify us in concluding that in a fruit-body of *Coprinus Rostrupianus*: (1) there are only two sexes, 50 per cent. of the spores being of one sex and 50 per cent. of the other and opposite sex; (2) every basidium bears two spores of one sex and two spores of the other and opposite sex; and (3) the hymenium bears only one sexual type of basidium.

As the work recorded here was nearing completion, the occurrence of *Coprinus* species the strains of which are bisexual was announced by both Vandendries and Brunswik. Thus these two investigators and the writer, working independently, all discovered bisexual *Coprini* simultaneously.

Vandendries (20), in a paper in 'La Cellule', showed that each strain of *Coprinus radians*<sup>1</sup> sexually has only two kinds of spores. His conclusions were based on two sets of experiments in which he made all possible crossings: (1) between twenty-three monosporous mycelia derived from one fruit-body, and (2) between twenty-five monosporous mycelia derived from another fruit-body. In the first set of experiments the spores without exception proved to be of only two sexes. In the second set twenty-four out of twenty-five spores also proved to be of only two sexes, but a twenty-fifth spore reacted positively with all the other spores, thus being anomalous in its sexual behaviour.

Vandendries, in his work on *Coprinus radians*, did not analyse the sexual reactions of the four spores of individual basidia; but it seems very probable that, had he done so, he would have obtained results similar to those recorded here for *C. Rostrupianus*, i. e. he would have found that each basidium bears two spores of one sex and two of another and opposite sex.

Vandendries, as already mentioned, met with an exceptional spore in his second set of experiments. He states that the spore was derived from the same fruit-body as the other twenty-four spores, but he admits that it behaved sexually as though it had come from another fruit-body belonging to a different sexual strain. In the experiments recorded in Table III with twenty-eight spores derived from the basidia of a single fruit-body of *Coprinus Rostrupianus* there was *not one single exception*, so that the evidence for bisexuality is even more satisfactory for *C. Rostrupianus* than for *C. radians*.

The sexual reactions of *Coprinus comatus*, *C. curtus*, *C. deliquescens*, *C.*

<sup>1</sup> *Coprinus radians* is a synonym for *C. domesticus*. Vide A. H. R. Buller, *Researches on Fungi*, vol. iii, pp. 41-2, 1924.

*ephemerus*, *C. radians*, and *C. velaris*, as determined by Brunswik (3), are similar to those of *C. radians* as determined by Vandendries, and to those of *C. Rostrupianus* as determined by the writer. However, for none of these species has Brunswik so far given us individual basidial analyses such as those of Tables II and III. Brunswik's theoretical conceptions differ from those of Kniep in that he explains the reactions between paired mycelia as due to the presence or absence of sterility factors rather than of sex factors.

Segregation of the sex factors in the basidia of *Coprinus Rostrupianus* results, as we have seen, in the production of two sexually different kinds of

TABLE IV.

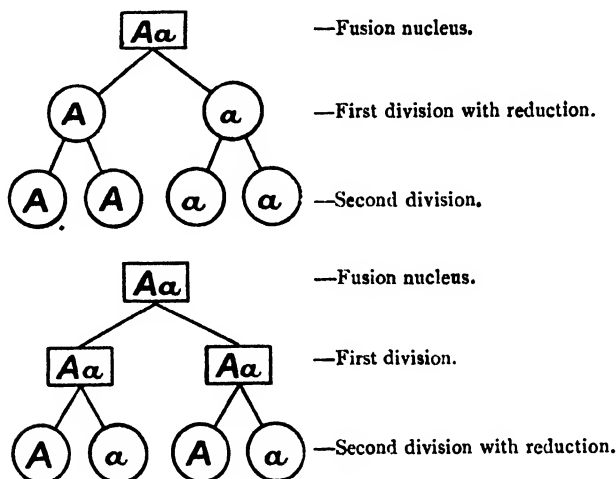
All Possible Pairings of Four Monosporous Mycelia derived from the Four Spores of a Single Basidium, with a Mendelian Interpretation of the Results.

		A		a	
		1	4	2	3
A	1	-	-	+	+
	4	-	-	+	+
a	2	+	+	-	-
	3	+	+	-	-

spores or haploid genotypes. Just as Kniep for *Schizophyllum commune* and Hanna for *Coprinus lagopus* have assumed that quadrisexuality may be explained on the assumption that two pairs of Mendelian factors are involved in the determination of sex, (Aa) and (Bb), both being present in the fusion nucleus of the basidium, so we may perhaps explain the bisexuality of *C. Rostrupianus* by supposing that sex in each strain of this species is determined by the presence, not of two, but of one pair of Mendelian factors, which we may call (Aa). On this assumption, when segregation of the sex factors takes place during the two divisions of the fusion nucleus, each spore will come to carry one or other of the factors (Aa), but not both. For twenty-one basidia altogether it has been found that each basidium bears two spores of one sex and two of another and opposite sex. This is exactly what we should expect on our assumption. When segregation of sex factors takes place in a basidium, two spores should come to carry the (A) factor and two the (a) factor. If now we rewrite Table II, assigning the symbols (A) and (a) to spores of opposite sex, it will have the appearance shown in Table IV.



It is impossible to determine without the use of cytological methods, whether the reduction process in the basidium takes place with the first division of the fusion nucleus or with the second; for in either case, as shown below, there would be produced two spores of one sex and two spores of the other and opposite sex.



TEXT-FIG. 17. Two possible modes of reduction in the basidium of *Coprinus Rostrupianus*.

Basidial dimorphism, or the presence of long and short basidia, as pointed out by Buller (5), occurs in many species of *Coprinus*; and it is also characteristic of *C. Rostrupianus*. It seemed possible that, when spore-tetrads were being removed from the hymenium of the fungus by means of the coverglass-contact method, the cover-glass might have touched the long basidia only. In order to find out whether or not the spores of the short basidia behave sexually like those of the long basidia, a general spore-deposit was obtained to which both long and short basidia had contributed: a slide was set under a mature fruit-body and a thin dry spore-deposit was collected. Nine of the spores were then sown separately in the culture medium, and the nine mycelia which resulted were then crossed with one another in all possible ways. The results of the crossings are embodied in Table V.

It will be observed from an inspection of the table that, from their reactions, mycelia Nos. 1, 2, 6, 8, and 9 are of one sex, and Nos. 3, 4, 5, and 7 are of another and opposite sex. If now we rearrange Table V so as to place like mycelia together and assign to each of the two groups of mycelia Mendelian symbols, we obtain Table VI.

An inspection of Table VI shows still more clearly than that of Table V that the nine mycelia fall into two opposite groups, those mycelia of like sex when paired remaining in the haploid condition, and those of

TABLE V.

*All Possible Pairings of Nine Monosporous Mycelia derived from Nine Spores collected from a General Spore-deposit.*

	1	2	3	4	5	6	7	8	9
1	-	-	+	+	+	-	+	-	-
2	-	-	+	+	+	-	+	-	-
3	+	+	-	-	-	+	-	+	+
4	+	+	-	-	-	+	-	+	+
5	+	+	-	-	-	+	-	+	+
6	-	-	+	+	+	-	+	-	-
7	+	+	-	-	-	+	-	+	+
8	-	-	+	+	+	-	+	-	-
9	-	-	+	+	+	-	+	-	-

TABLE VI.

*Table V rearranged, with a Mendelian Interpretation of the Results.*

		<i>A</i>					<i>a</i>			
		1	2	6	8	9	3	4	5	7
<i>A</i>	1	-	-	-	-	-	+	+	+	+
	2	-	-	-	-	-	+	+	+	+
	6	-	-	-	-	-	+	+	+	+
	8	-	-	-	-	-	+	+	+	+
	9	-	-	-	-	-	+	+	+	+
<i>a</i>	3	+	+	+	+	+	-	-	-	-
	4	+	+	+	+	+	-	-	-	-
	5	+	+	+	+	+	-	-	-	-
	7	+	+	+	+	+	-	-	-	-

unlike sex when paired yielding a diploid clamp-bearing mycelium. Furthermore, the Mendelian interpretation, which here involves the assumption that mycelia which are sexually alike repel one another, while mycelia

of unlike sexes attract one another or react positively, fits the reactions contained in the table; for it is clear that (A) fails to mate with (A), and (a) with (a), while (A) and (a) always give positive results. Since, doubtless owing to the method of obtaining the spores, some of the mycelia were derived from the spores of long basidia and other mycelia from the spores of short basidia, we may conclude that the dimorphism of the basidia is in no way linked with sexual phenomena, i. e. that both long and short basidia bear spores of two sexes and of two sexes only.

Vandendries (19) collected spores from the wild fruit-bodies of *Panaeolus campanulatus* and *P. separatus* and analysed their sexual reactions. He found that while any individual strain of *P. campanulatus* and *P. separatus* was in general 'bipolar', i. e. bisexual, there were numerous exceptions to this rule, a number of spores being 'hermaphrodite', i. e. reacting positively with both sexual groups. However, when he analysed the sexual reactions of spores derived from first-generation fruit-bodies reared in the laboratory from two spores of opposite sex, he found that the spores showed distinct sexual 'bipolarity' with few exceptions. Vandendries collected spores from wild fruit-bodies of *Coprinus radians*; but in this species, as we have seen, there was only one exception to the rule of bisexuality. Basing his views on these results, Vandendries offered the suggestion that, in wild fruit-bodies, the two sexes are not strictly segregated from one another ('dans une sporée sauvage les sexes ne sont pas rigoureusement opposés'). He supposed that, in nature, fruit-bodies often arise from mycelia derived from many diploid mycelia, each mycelium owing its origin to the union of two haploid mycelia produced by two spores of opposite sex; and he believed that this accounts for the irregularity of his experimental results with the two species of *Panaeolus*. On the other hand, to account for the almost uniform 'bipolarity' with spores obtained from wild fruit-bodies of *Coprinus radians*, he supposed that these wild fruit-bodies happened each to have been derived from a single pair of spores (20).

Having observed that a considerable percentage of the monosporous mycelia of *Coprinus radians* in course of time pass spontaneously from the haploid to the diploid (clamp-bearing) condition, Vandendries has recently expressed the view that each of the wild fruit-bodies, to which reference has just been made, may have been derived not from two spores of opposite sex but from a single spore (21).

Against Vandendries's theory that wild fruit-bodies are derived from many diploid mycelia various arguments may be adduced. Firstly, Brefeld (2) has shown that a fruit-body of *Coprinus stercorarius* or *C. lagopus* arises from a single hypha bearing clamp-connexions, and that, having had this origin, the fruit-body develops to maturity. Brefeld's observations on *C. stercorarius* have been confirmed by the writer. There is every reason to suppose that all *Coprinus* fruit-bodies spring from a single diploid hypha,

and that what happens in Petri dishes in the laboratory also happens in nature. Secondly, Hanna analysed the sexual reactions of spores of *Coprinus lagopus* (a species with quadrisexual strains) collected from six wild fruit-bodies derived from six geographically different places, and found that the spores in each case fell into four groups with perfect regularity (8). Had any one of these fruit-bodies been derived from several diploid mycelia, the chances are that the mycelia contributing to the formation of the fruit-body might well have belonged to two or more sexual strains (which are perfectly fertile *inter se*), and this would have been revealed by the increase in the number of positive reactions when the monosporous mycelia obtained from the spores of the wild fruit-body were crossed. Since there was no such increase in positive results, the evidence points to each of Hanna's six wild fruit-bodies having had a bisporous origin. Thirdly, two of the fruit-bodies of *Coprinus Rostrupianus* used for the present investigation were derived from sclerotia grown from mycelia which were produced from a mixture of many spores of two perfectly interfertile sexual strains sown thickly together in the laboratory. An analysis of the sexual reactions of the spores derived from each of the two fruit-bodies showed that the spores of each fruit-body fell into sexually opposite groups without exception. If we represent the two sex factors of one strain as (A) and (a), and the two sex factors of the other strain as (A<sup>1</sup>) and (a<sup>1</sup>), then the possible unions would be as follows: (Aa), (AA<sup>1</sup>), (Aa<sup>1</sup>), (A<sup>1</sup>a), (A<sup>1</sup>a<sup>1</sup>), (aa<sup>1</sup>). A compound mycelium composed of two or more of these six diploid mycelia would contain three or four of the factors (A), (a), (A<sup>1</sup>), (a<sup>1</sup>); so that, if a compound mycelium of the type indicated were to enter into the composition of a single fruit-body, that fruit-body would bear spores of three or four different kinds; and, on making the crosses, the sexes of the spores would fall not into two groups but into three or four. Thus our three arguments all point to the conclusion that a wild or cultivated fruit-body of any species of *Coprinus*, *Panaeolus*, &c., is derived from a single diploid mycelium which owes its origin to the union of only two haploid mycelia which have been developed from two spores of opposite sex.

It has been shown by Kniep for *Schizophyllum commune* (12), Vandendries for *Panaeolus campanulatus* (19) and *Coprinus radians* (20), Hanna for *Coprinus lagopus* (8), and Brunswik for *Coprinus comatus*, *C. picaceus*, *C. niveus*, *C. lagopus*, *C. finetarius* and *C. Friesii* (3) that in each of these species there are different sexual strains. Thus in a quadrisexual species, e. g. *Schizophyllum commune*, and *Coprinus lagopus*, in each strain there are four sexually different kinds of spores, yet the four kinds of spores of one strain differ in their sexual constitution from the four kinds of any other strain, with the result that any spore of one sexual strain is perfectly fertile with any spore of any other sexual strain. Brunswik reports the finding of twenty-seven such perfectly interfertile strains in *Coprinus finetarius* (3).

*Coprinus Rostrupianus* was found to resemble the Hymenomycetes just mentioned in possessing sexual strains which *inter se* are perfectly fertile, any spore of one strain producing a diploid clamp-bearing mycelium with any spore of another strain. Eight monosporous mycelia derived from one wild fruit-body were crossed with eight other monosporous mycelia derived from a second wild fruit-body. As shown by the results embodied in Table VII

TABLE VII.

*All Possible Pairings between Eight Monosporous Mycelia of a Fruit-body of One Sexual Strain and Eight Monosporous Mycelia of a Fruit-body of Another Sexual Strain.*

		A'				a'			
		1	2	3	4	5	6	7	8
A	I	+	+	+	+	+	+	+	+
	II	+	+	+	+	+	+	+	+
	III	+	+	+	+	+	+	+	+
	IV	+	+	+	+	+	+	+	+
a	V	+	+	+	+	+	+	+	+
	VI	+	+	+	+	+	+	+	+
	VII	+	+	+	+	+	+	+	+
	VIII	+	+	+	+	+	+	+	+

complete interfertility resulted. If (A) and (a) be used as symbols for the spores of opposite sex of one of the two fruit-bodies, then we may use the symbols (A<sup>1</sup>) and (a<sup>1</sup>) for the spores of opposite sex of the other fruit-body, as shown in the table, where it has been arbitrarily assumed that in one fruit-body the spores I-4 were of one sex and 5-8 of the other sex, and that in the other fruit-body the spores I-IV were of one sex and V-VIII of the other sex.

As an illustration of the danger of drawing false conclusions when these are based on an insufficient number of observations, an experiment may be mentioned in which ten monosporous mycelia of one fruit-body were crossed in all possible ways, with the result shown in Table VIII. It will be seen that in no case did clamp-connexions develop, showing that all ten spores were of one sex.

Now the chances that ten spores picked successively at random from

a deposit of equal numbers of spores of two sexes shall all be of the same sex is, according to the law of probability,  $1 : 1,024$ ; and it seemed possible that the fruit-body from which the spores had been derived might have originated from a sclerotium produced by the mycelium of a single spore, in which case, as would be expected from the previous experience of Kniep (11) and Hanna (8), all the spores of the fruit-body should be of one sex. But

TABLE VIII.

*All Possible Pairings of Ten Monosporous Mycelia derived from Ten Spores of a Single Fruit-body.*

	1	2	3	4	5	6	7	8	9	10
1	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—

that the fruit-body was not of monosporous origin and did actually have spores of two different sexes was shown by sowing many of the spores together and obtaining therefrom a diploid clamp-bearing mycelium. Evidently, therefore, the selection of ten spores of one sex was a remarkable chance occurrence.

About October 15 twenty-eight spores derived from seven basidia were successfully germinated. In the third week of October the four mycelia resulting from the four spores of each basidium were crossed in all possible ways. Towards the end of October the plates were examined for clamp-connexions, and it was found that each basidium had borne two spores of one sex and two of another and opposite sex (Table III). It was clear that all the twenty-eight mycelia were unisexual. In order to find out whether or not this unisexuality would be retained by the mycelia indefinitely, on November 3 the mycelia were transferred from the agar plates to sterilized

quart jars half filled with horse-dung and plugged with cotton-wool. On December 17, i. e. eight weeks after the spores had been sown, pieces of the twenty-eight mycelia were transferred to as many agar plates. About December 24 all the plates were examined with the microscope. Two of the mycelia showed no growth. The other twenty-six mycelia all proved to be devoid of clamp-connexions. Therefore these twenty-six mycelia had retained their unisexual character for about nine weeks.

On December 17 transfers of the twenty-eight mycelia were made not only to agar plates, as recorded above, but also to sterilized pint jars half filled with dung and plugged with cotton-wool. As some of the mycelia failed to grow, a few second inoculations were made. Finally twenty-six of the mycelia grew well, while two failed to develop. On January 21 the twenty-six mycelia were transferred from the dung to as many agar plates. About February 7 all the plates were examined with the microscope. It was now found that while twenty-one of the mycelia were still unisexual, the other five mycelia exhibited clamp-connexions, thereby showing that they had passed from the unisexual (haploid) condition to the bisexual (diploid) condition. Thus between the ninth and the sixteenth week of their existence five mycelia had changed spontaneously from the haploid to the diploid condition.

On January 31 transfers of the twenty-six mycelia were made not only to agar plates, as recorded above, but also to sterilized quart jars half filled with dung and plugged with cotton-wool. Two of the mycelia did not grow: on February 7 one of these had been found to be haploid and the other diploid. On March 28 the twenty-four living mycelia were transferred from the dung to as many agar plates. About April 4 all the plates were examined with the microscope. It was found that the four mycelia which had become diploid by February 7 still continued to be diploid, and that of the other twenty mycelia which on February 7 had all been haploid nine had now become diploid, the total now being: eleven haploid and thirteen diploid. Thus between the sixteenth and the twenty-fourth week of their existence, nine mycelia had changed spontaneously from the haploid to the diploid condition.

Summarizing the series of observations just recorded, we have clear evidence that, in the course of six months, of twenty-five monosporous mycelia continuously cultivated eleven mycelia retained their haploid condition, while fourteen mycelia, or 56 per cent. of the whole, changed spontaneously from the haploid to the diploid condition.

As already indicated in the introduction, the sexual change in the monosporous mycelia of *Coprinus Rostrupianus* here recorded resembles that found by Vandendries (20) for the monosporous mycelia of *C. radians*. Of his mycelia twenty-seven changed from the haploid to the diploid condition in the course of six months.

Vandendries (21) has advanced the theory that all species of *Coprinus*, &c., are at first heterothallic: i. e. that the spores of the so-called homothallic species, as well as those of heterothallic species, are all unisexual, and that the mycelia which the spores produce are at first also unisexual. He suggests that in a homothallic species, e. g. *Coprinus sterquilinus*, the mycelia change from the haploid to the diploid condition at a very early stage in their development, whilst in a heterothallic species the change is delayed and may take place only after several weeks or months, as actually occurs with *C. radians*. Vandendries therefore regards *C. sterquilinus* not as homothallic and *C. radians* not as heterothallic, but both of them as *hetero-homothallic*.

Let us suppose that in *Coprinus radians* or *C. Rostrupianus* two haploid mycelia bearing the sex-factors (A) and (a) are mated and give rise to a diploid mycelium from which a fruit-body is produced. This fruit-body will yield 50 per cent. of spores with the (A) factor and 50 per cent. with the (a) factor. Let us suppose further that a mycelium which has come from a spore with the (A) factor spontaneously becomes transformed into a diploid mycelium. We do not as yet know whether the two sexes represented in each pair of nuclei in the diploid phase of this mycelium are identical with the parent sexes and can be represented by the symbols (A) and (a), or whether the two sexes differ from the parental sexes and must therefore be represented by two new symbols, say (A<sup>1</sup>) and (a<sup>1</sup>), in which latter case we should be obliged to explain the spontaneous change from the haploid to the diploid condition by the theory of mutation. To solve the problem of the nature of the two sexes in our diploid mycelium, it would be necessary to obtain a fruit-body from the diploid mycelium, collect the spores therefrom, germinate them, and back-cross their mycelia with portions of the original haploid parental mycelia or their sister mycelia. If the species is hetero-homothallic, the crossings should yield only two groups of mycelia of opposite sex; but if the spontaneous change to the diploid condition has been due to mutation, there should be three or four groups corresponding to the factors (A), (a), (A<sup>1</sup>), and (a<sup>1</sup>). The true explanation of the spontaneous change from the haploid to the diploid condition in *C. radians* and *C. Rostrupianus*, therefore, can be given only after further critical experiments have been made.

Finally, we can ask: Do the wild fruit-bodies of *Coprinus Rostrupianus* originate from a diploid mycelium which has been produced by the union of two haploid mycelia of opposite sex, or from a diploid mycelium which has come into existence from a single haploid mycelium by a spontaneous change? The writer is of the opinion that, owing to the large number of spores liberated by each fruit-body and their prevalence on the grass of the meadows where the fungus exists, it is likely that, as a rule, a dung-plot will contain not one spore of *C. Rostrupianus* but many spores, thus giving



ample opportunity for diploid mycelia to be rapidly produced by the union of haploid mycelia of opposite sex. Probably, therefore, the great majority of wild fruit-bodies of *C. Rostrupianus*, and perhaps all of them, arise individually not from a single haploid mycelium which, in the course of many weeks, has spontaneously become diploid, but from two haploid mycelia of opposite sex which have fused and formed a diploid mycelium.

#### V. SUMMARY.

1. *Coprinus Rostrupianus* is heterothallic, in that its monosporous mycelia during the first few weeks of their existence are all unisexual, half of them being of one sex and the other half of another and opposite sex.

2. Of twenty-five monosporous mycelia, which were cultivated continuously for six months, eleven remained haploid while fourteen, or 56 per cent. of the whole, spontaneously became diploid.

3. *C. Rostrupianus* resembles *C. radians*, as investigated by Vandendries, in that all the spores and young mycelia of a single sexual strain belong to one of two sexes and in that, in the course of some months, many of the monosporous mycelia change spontaneously from the haploid to the diploid condition. Whether or not both of these species should be regarded as hetero-homothallic can only be decided by further investigation.

4. In any individual strain of *C. Rostrupianus* only one type of basidium exists. Each basidium bears one pair of spores of one sex and another pair of spores of another and opposite sex. In any spore-deposit half the spores are of one sex and the other half of another and opposite sex.

5. It is impossible to decide by the experimental methods used whether the reduction process in the basidium takes place with the first or with the second division of the fusion nucleus.

6. Dimorphism of the basidia is not linked with differences in their sexual constitution. Both long and short basidia bear spores of both sexes.

7. Both haploid and diploid mycelia of *C. Rostrupianus* produce sclerotia; but, whereas sclerotia of diploid origin fruit perfectly, those of haploid origin give rise to infertile mycelia or to fruit-body rudiments which soon cease to develop.

8. It seems probable that every wild fruit-body of *C. Rostrupianus*, *C. lagopus*, and many other heterothallic Agaricineae develops from a single diploid mycelium formed by the union of two haploid mycelia of opposite sex, and not from several or many diploid mycelia.

9. *C. Rostrupianus* comprises sexually different strains which show complete interfertility, so that, while each sexual strain is bisexual, the species as a whole must be regarded as multisexual.

The foregoing investigations were carried out in the Department of Botany of the University of Manitoba, partly during the tenure of the

Hudson's Bay Fellowship for 1923-4, and partly during the tenure of a Fellowship granted by the Canadian Honorary Advisory Council for Scientific and Industrial Research for 1924-5. I desire to acknowledge my debt to Professor A. H. Reginald Buller for suggesting the problem and for giving me the benefit of his wide knowledge and experience during the progress of the work.

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## EXPLANATION OF PLATE VI.

Illustrating Miss Dorothy Newton's paper on *Coprinus Rostrupianus*.

All figures are those of *Coprinus Rostrupianus*.

Fig. 1. Two sclerotia found at Winnipeg in old cow-dung, photographed when dry and shrunken. Natural size.

Fig. 2. A group of nine moist and fully swollen sclerotia showing variation in form and size, grown from polysporous (diploid) mycelia on sterilized horse-dung in the laboratory. Natural size.

Fig. 3. The production of fruit-bodies from sclerotia found in old cow-dung. Photograph taken three weeks after the sclerotia had been placed on moist sand in the laboratory. On the left, a single sclerotium bearing the rudiment of a fruit-body. On the right, two sclerotia clinging together, each bearing a fruit-body which is about to elongate its stipe and expand its pileus. Natural size.

Fig. 4. The same two sclerotia and two fruit-bodies as those shown in Fig. 3, one day older. The stipes are now fully elongated. The pilei, which have become campanulate and are now shedding spores, still have their gills interlocked by long cystidia which could be seen with the naked eye. Natural size.

Fig. 5. Spores which settled on a glass slide placed beneath a pileus, photographed dry.  $\times 700$ .

Fig. 6. A spore-deposit on a cover-glass, obtained by the coverglass-contact method, showing five spore-tetrads, each tetrad being made up of the four spores of a single basidium.  $\times 100$ .



Hath coll.

NEWTON-COPRINUS.



# Chemical Studies in the Physiology of Apples.

## V. Methods of Ash Analysis, and the Effect of Environment on the Mineral Constitution of the Apple.

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### INTRODUCTION.

IT has been shown in work published by Haynes (15), Archbold (3), and Carré (5), that apples of the same variety grown on different soils show considerable variation in such chemical properties as acidity, nitrogen content, and pectin content, as well as in commercial qualities such as keeping property and flavour. Very little information, however, is available as to the corresponding variation in mineral constituents; accordingly an investigation of the ash of apples grown on different soils has been undertaken. Some work has also been carried out on the mineral constituents of the same variety of apples from trees grown on different stocks.

It is evident that the determination of the ash constituents of apples is of little value unless the significance of any differences can be estimated. The probable error of the estimations of the ash constituents of Bramley Seedling apples from one locality has accordingly been carefully determined from the mineral analyses of no less than thirty separate apples. These determinations were also part of a general chemical study of individual apples, other aspects of which are being studied in this laboratory by Dr. D. Haynes and Miss H. Archbold; this work is to be published later.

Preliminary to the investigation reported in this paper, an examination of methods of mineral analysis suitable for small amounts of material was found to be necessary, as apples contain only about 0.2 per cent. of mineral matter. The mineral constitution of 100 grm. of freshly cut-up apple (Lane's Prince Albert) is roughly as follows: potash ( $K_2O$ ) 0.8–0.12 grm.,

lime ( $\text{CaO}$ ) 0.002–0.005 grm., magnesia ( $\text{MgO}$ ) 0.006–0.008 grm., phosphate ( $\text{P}_2\text{O}_5$ ) 0.018–0.024 grm., iron ( $\text{Fe}_2\text{O}_3$ ) 0.0004–0.0007 grm.; considerable amounts of carbonate and sulphate are also found, together with a little silica and traces of alumina, sodium, and chloride. In order to perform a sufficient number of analyses to give a significant result it was necessary to avoid very laborious methods of analysis, even at some sacrifice of accuracy.

## I. METHODS OF ANALYSIS.

In order to obtain a representative sample twenty to thirty apples were taken. These were peeled, cored, cut up and thoroughly mixed, and then dried in an electric air oven at  $100^\circ\text{C}$ . for thirty-six hours, and their dry weight was determined at the same time, as described by Archbold (2).

The dried apple was then ground up in a porcelain mechanical mortar, heated at  $100^\circ\text{C}$ . for three hours, and poured into bottles which were sealed with paraffin wax. In this condition the material will keep indefinitely, and may be analysed at leisure. For the process of ashing, from 2 to 10 grm. of dried material were used. The material was weighed correct to the nearest centigram by difference, as it is very hygroscopic, and then placed in a weighed platinum dish, and heated very gently over a Bunsen flame until it was charred. The dish was then placed in a silica muffle furnace, of which the temperature was kept below redness; high temperatures cause loss of sulphate and of potassium salts. At intervals of about an hour during heating the dish was taken out of the furnace and the contents ground up with a little water by means of a flat-headed glass rod. The mixture was then evaporated to dryness and the dish was replaced in the oven. This greatly accelerates the process of ashing, which can be carried out thus in about six hours. When all the organic matter had been oxidized the dish was placed in a desiccator over sulphuric acid for eight to ten minutes and then weighed as rapidly as possible. The dish was replaced in the muffle furnace and weighed again; this was repeated until it was constant in weight. When ashed in this manner results were obtained which usually agreed to within two or three milligrams, or about four per cent. of the ash. The most serious source of error is from the loss of sulphate in ashing, and there is also a possibility of gain from the flue gases. Under standard conditions the results obtained agree with each other, but there may be a constant error which differs slightly according to the proportions of sulphur present in the original material. The matter requires to be investigated farther. For the accurate estimation of sulphate in the material, special methods of ashing are necessary, which are described by Stockholm and Koch (28) and Barlow (4).

Estimations of lime, potash, phosphate, magnesia, and iron were carried out on the ash. These substances, with the exception of phosphate

and magnesia, which could be estimated in the same solution, were estimated in separate amounts of ashed material; the weight of the ash was determined in each case, so that six or eight determinations of ash were always made. Before the ash was used for the estimations, silica was rendered insoluble by evaporating the ash to dryness with concentrated hydrochloric acid, except in the estimation of iron, when sulphuric acid, or nitric acid and a few drops of hydrofluoric acid, were used. Thus in the descriptions of individual estimations the term 'residue' is used to denote the ash in which the silica had been rendered insoluble. The accuracy of the methods of estimation was determined by using standard solutions containing approximately the same quantities of the minerals that were to be estimated. All the flasks, pipettes, and burettes used in the volumetric work were calibrated and very carefully washed. The filter-papers used were No. 40 Whatman (7 cm.).

#### *Estimation of Potassium.*

The potash was precipitated from an aqueous solution of the 'residue' with perchloric acid, and was dried and weighed as perchlorate, as described by Morris (19) and Mellor (18). A Monroe crucible was used for the estimation. To avoid the solution of the potassium perchlorate precipitate in the wash liquid of ninety-eight per cent. alcohol containing 0.2 per cent. perchlorate acid, it was saturated with potassium perchlorate and filtered before use. The temperature was kept as low as possible during the filtering and washing, since the solubility of potassium perchlorate increases rapidly with rise of temperature.

Calcium sulphate is slightly soluble in water and insoluble in alcohol, and hence tends to be carried down with the perchlorate precipitate, unless sulphate is removed from the solution before the estimation of the potash. When very small amounts of potash were to be estimated, and when greater accuracy was required, sulphate was removed by the addition of a slight excess of two per cent. barium chloride solution to the boiling dilute solution of the residue. The mixture was filtered and washed after thirty-six hours, and the potash was estimated in the filtrate. When the sulphate was not removed from the solution it was necessary to apply a correction. Experiments on a series of standard solutions showed that the presence of sulphate did not affect the estimation if excess of perchlorate acid had been used, but in the presence of calcium as well as sulphate the results were always too high, calcium sulphate being precipitated with the perchlorate precipitate. Therefore, by estimating the sulphate in the precipitate a correction can be applied.

This can rapidly be done by a nephelometric method. In this method the perchlorate precipitate, after weighing, was washed through the crucible with hot water and diluted to 100 c.c., or 50 c.c. for smaller amounts, and



cooled, 50 c.c. were taken, and 2 c.c. of two per cent. barium chloride were added to precipitate barium sulphate as a cloud. The solution was stirred for two to three minutes, and the turbidity was compared in a nephelometer (see phosphate estimation) with that given by a standard solution of sulphate, and the correction applied.

TABLE II.

*Effect of Sulphates of Potassium and Calcium on the Estimation of Potassium.*

<i>Solution.</i>	<i>Found grm. K.</i>	<i>Grm. K present.</i>	<i>Difference (per cent.).</i>
KCl	0.0712	0.0717	0.7
KCl 0.0528 grm. $K_2SO_4$	0.0556	0.0566	1.8
KCl 0.042 „ $K_2SO_4$	0.0622	0.0629	1.1
KCl 0.018 „ $CaSO_4$	0.0417	0.0363	13.0

TABLE III.

*Comparison between Corrected and Uncorrected Values of Estimations of Potash in the Presence of Calcium.*

<i>Solution.</i>	<i>Found (grm.)</i>		<i>Difference (per cent.).</i>	
	<i>Uncorrected.</i>	<i>Corrected.</i>	<i>Uncorrected.</i>	<i>Corrected.</i>
KCl, $K_2SO_4$ , and $CaSO_4$ containing 0.069 grm. K	0.0720	0.0687	4.3	0.4
	0.0715	0.0685	3.6	0.7
	0.0706	0.0680	2.3	1.4
	0.0668	0.0641	2.8	1.5
KCl and $CaSO_4$ contain- ing 0.0651 grm. K	0.0676	0.0643	3.0	1.2
	0.0665	0.0640	2.1	1.7
	0.0667	0.0642	2.5	1.4

*Estimation of Lime.*

Alport's method (1) slightly modified was used, the precipitate of calcium as oxalate being titrated with standard permanganate.

*Method.* The 'residue' was heated with six drops of glacial acetic acid and 0.5 grm. of ammonium acetate to remove any free hydrochloric acid, and 8.0 c.c. of water. The liquid was then filtered, and washed four or five times with water acidified with two drops of glacial acetic acid, making 25–30 c.c. in all. Calcium oxalate was precipitated by the addition of ten drops of hot ammonium oxalate to the boiling liquid; the mixture was then stirred and left to stand all night. Next day it was filtered slowly under slight pressure through a Monroe crucible of thimble size, and washed with cold water until free from oxalates. The Monroe crucible was then fitted to a small flask, into which the calcium oxalate precipitate was dissolved by pouring through from 12 to 15 c.c. of hot dilute sulphuric acid (10–12 c.c. water and fifteen drops of 1:10 sulphuric acid). The flask was heated to 60° C. and the oxalate solution was titrated with N/50 potassium

permanganate. When the pink colour was permanent, the Monroe was further washed with about 2 c.c. dilute sulphuric acid into the flask, which was again heated to 60° C.; if necessary more permanganate was added, and the process was repeated until further washing ceased to alter the colour.

*Standard Solutions.* The standard solutions of sodium oxalate and potassium permanganate were made up to N/5 and diluted immediately before use.

A series of determinations were carried out on a solution containing a known weight of calcium.

TABLE IV.

*To show the Degree of Accuracy of the Estimation of Calcium.*

<i>Solution.</i>	<i>Titration in c.c. N/50 KMnO<sub>4</sub>.</i>	<i>Found CaO in mg.</i>
25 c.c. of solution of	6.35	3.5
Iceland spar in 10 c.c.	6.09	3.4
acetic acid diluted to	6.12	3.4 mean
500 c.c. = 3.75 mg.	6.47	3.6
CaO	6.42	3.5

#### *Estimation of Magnesium.*

Owing to the small percentage of magnesium present, gravimetric methods of estimation were found to be unsatisfactory, and a nephelometric method was also found to be unreliable. The method of Tisdall and Kramer was then tried and proved to be very satisfactory (24).

TABLE V.

*To show the Degree of Accuracy of the Magnesium Estimation.*

<i>Solution.</i>	<i>Mg found (mg.).</i>
MgSO <sub>4</sub> solution	1.5
MgSO <sub>4</sub> + A	1.2
„ + B	1.2
„ solution	2.5
„ + A	2.6
„ + B	2.6
MgSO <sub>4</sub> , 7H <sub>2</sub> O solution.	
A. { 10 c.c. Iceland spar solution in acetic acid containing 1.5 mg. CaO + 10 c.c. K <sub>2</sub> SO <sub>4</sub> { solution containing 4.1 mg. K <sub>2</sub> .	
B. A + 5 c.c. KH <sub>2</sub> PO <sub>4</sub> solution containing 0.5 mg. P <sub>2</sub> O <sub>5</sub> .	
2 c.c. of concentrated hydrochloric acid were present in each estimation, and the volume of the whole was made up to 30 c.c.	

*Method.* The 'residue' was dissolved in 2 c.c. of hydrochloric acid and 25 c.c. of water and filtered. Calcium was precipitated as oxalate without being removed, and then the magnesium as ammonium magnesium phosphate. After standing for twelve hours the mixture was filtered; the precipitate and the filter-paper were mixed with water in the beaker and excess

of N/10 hydrochloric acid was added, and the phosphoric acid set free was titrated with N/10 sodium hydroxide to sodium dihydrogen phosphate, using cochineal as indicator.

*Standard Solutions.* The standard hydrochloric acid was made up by distilling 1 : 1 density hydrochloric acid and using the constant boiling hydrochloric acid as described by Hallett and Bonner (10).

Table V, p. 133, shows the accuracy of the method; it is rapid, and six or eight estimations may be carried out in four hours.

#### *Estimation of Phosphate.*

The intensity of the precipitate with molybdic acid-quinine reagent was compared in the nephelometer with that given by a standard under the same conditions. The method of Pouget and Chouehak (20) in use at the Imperial College of Science for soil analysis was employed.

*Method.* The 'residue' was taken up with 1 : 1 hydrochloric acid, digested on a steam bath to ensure complete solution, filtered and washed with water. The filtrate was made up to 100 or 250 c.c. according to the amount of phosphate present. For each determination 5 c.c. or 10 c.c. of this solution were placed on a Nessler cylinder and diluted to 46 c.c., 2 c.c. of 1.12 nitric acid were added, and then 2 c.c. of molybdic acid-quinine reagent. The cylinder was corked and allowed to stand for 15–20 minutes, and then the reading of the nephelometer was taken and compared with that given by a standard phosphate solution similarly treated. The dilution of the standard and of the test solution was arranged so as to give a reading of between 17 and 27 c.c. of the nephelometer.

*Standards.* A standard phosphate solution was made up of potassium hydrogen phosphate ( $K_2HPO_4$ ) containing about 0.1 mg. per 10 c.c.; it was found convenient to make up a solution ten times as strong and dilute. The molybdic acid-quinine reagent contained one grm. of quinine hydrochloride dissolved in dilute nitric acid, to which was added a solution of 40 grm. of ammonium molybdate dissolved in water and a little ammonia and then 500 c.c. of nitric acid (density 1.2), and the whole was diluted to one litre. It was kept in the dark and filtered if necessary before use. By using quinine hydrochloride instead of quinine sulphate a great deal of time was saved, as the sulphate had not to be removed from the reagent with barium hydroxide, and hence it was not necessary to remove the sulphate from the test solution; however, the reagent did not keep quite so well.

The *nephelometer* was constructed out of a wooden box divided into two compartments by a piece of ground glass. In one compartment was fitted an electric bulb; the light of which coming through the glass served to illuminate the other compartment in which was the nephelometer tube, standing on a card having 256 divisions per square inch. The tube was graduated in cubic centimetres and the bottom was of flat plate glass. The

cloudy mixture to be tested was poured slowly into the tube until, on looking down from the top, the divisions on the card could no longer be distinguished. The volume of liquid was then read through a window in front of the box.

As it was found difficult to pour the liquid into the nephelometer tube sufficiently slowly to give an accurate final reading, a slight excess was added and the reading taken, and then a few drops of the cloudy liquid were removed with a tube until the divisions were just seen again, and drop by drop the liquid was added until the divisions were obscured; this gave the final reading. At least five readings were taken of each solution. The method is accurate to within about 5 per cent., and it is very rapid, as a large number of determinations can be carried out consecutively.

TABLE VI.

*To show the Accuracy of the Phosphate Estimation using Various Volumes of Standard Solutions.*

<i>Volume of Phosphate Solution (containing 0.01 mg. <math>P_2O_5</math> per c.c.).</i>	<i>Neph. Reading in c.c.</i>	<i>Found <math>P_2O_5</math> (mg.).</i>	<i>Difference (per cent.).</i>
9.0 c.c.	24.0	0.088	2.2
9.5 c.c.	22.0	0.092	3.3
10.0 c.c.	21.25	—	—
10.5 c.c.	20.5	0.103	2.0
11.0 c.c.	20.5	0.109	1.0

The reading of 21.25 c.c., corresponding to 0.1 mg. of phosphate, was used as standard.

#### *Estimation of Iron.*

As the amount of iron present in the apple is very small, the thiocyanate colorimetric method was found to be the most suitable. The colour produced under exactly similar conditions in the ash solution and in a standard solution was compared. The method is described by Snell (21) and Mellor (18).

*Method.* The 'residue' was dissolved in 1:10 sulphuric acid, oxidized by boiling with a few drops of nitric acid, filtered and washed with water into a 100 c.c. flask. For each determination 5 c.c.—25 c.c. of the solution were taken in a 50 c.c. Nessler cylinder, and 5 c.c. of the diluted standard iron solution were placed in a similar Nessler cylinder. Both solutions were diluted to 45 c.c. with 1:25 sulphuric acid (by volume) and then 5 c.c. of thiocyanate solution were added to each. The two colours were matched by adding standard iron solution drop by drop from a burette into the paler of the two solutions, stirring after each addition and keeping the volume in the two cylinders equal by the addition of water. From the amount of iron present in the standard, that in the test solution could be calculated.

*Standard Solutions.* The thiocyanate solution contained 24 grm. of pure potassium thiocyanate dissolved in 250 c.c. of water. The solution was kept in the dark and renewed frequently. A litre of iron solution, one cubic centimetre of which represented 0.0001 grm. of  $\text{Fe}_2\text{O}_3$ , was made up by dissolving 0.6303 grm. of ferric potassium alum ( $\text{Fe}_2(\text{SO}_4)_3 \cdot \text{K}_2\text{SO}_4 \cdot (24\text{H}_2\text{O})$ ) in water containing 5 c.c. of concentrated sulphuric acid; more dilute solutions would hydrolyse. For use a diluted standard was made up; 5 c.c. of the solution were transferred to a 100 c.c. flask, and 25 c.c. of a salt solution (see below) were added, and the mixture diluted to the mark with water. Of this 5 c.c. containing 0.025 mg. of  $\text{Fe}_2\text{O}_3$  (0.005 mg. per c.c.) were used for each estimation. If more than about 1.5–2.0 c.c. of the standard were required to match the colours the dilution of the standard was altered until it was similar to that of the test solution.

*Salt Solution.* As the presence of other salts slightly affects the colour, a solution containing approximately the same proportion of the salts that are present in the ash was made up, so that 25 c.c. contained about the same weights of the elements as were present in 100 c.c. of the test solution (that is, ash from 10 grm. of dried apple). The solutions contained 0.4 grm.  $\text{CaSO}_4$ , 2.5 grm.  $\text{K}_2\text{HPO}_4$ , 6.0 grm.  $\text{K}_2\text{SO}_4$ , and 0.6 grm.  $\text{MgSO}_4$ , dissolved in water and 5 c.c.  $\text{H}_2\text{SO}_4$  and diluted to 1 litre.

A few qualitative tests on the amount of sulphuric acid required were made, as McIlor (18) does not recommend the addition of any further sulphuric acid to the solution in the Nessler cylinders, while according to Snell (21) the solutions should be 'slightly acid', except in very dilute solutions, when he adds 10 c.c. of concentrated sulphuric acid. It was found that, under the conditions of the experiment, when less than 1 : 1000 parts of sulphuric acid was added to the solution in the Nessler cylinder only a faint yellow colour appeared, while 1 : 10 sulphuric acid caused the colour to disappear completely owing to decomposition of the thiocyanate; 1 : 25 or 1 : 20 sulphuric acid was found to be best.

This method is very accurate, and a number of determinations carried out using different volumes of the standard in most cases required the calculated volume of standard to match the colours, the error being less than 5 per cent. In order to discover the amount of iron introduced from the atmosphere and dust, a few grammes of cane sugar and 25 c.c. of the salt solution were ashed in a platinum dish and treated as for iron solution. The whole solution gave only a faint colour with thiocyanate solution, showing that the error introduced in ashing was negligible.

## II. ESTIMATION OF MINERAL CONSTITUENTS OF APPLES FROM DIFFERENT LOCALITIES.

It was doubtful whether analysis of the whole apple or of the juice would provide the better basis of comparison; accordingly some preliminary

work was begun on the latter. A series of analyses of juices of the same variety of apples expressed at different dates extending over a period of six months, gave such widely varying results,<sup>1</sup> that it was thought that the variation was probably due to the differences of acidity producing differences in the amount of salts extracted from the tissues. It was therefore decided that the analysis of the juice was unsuitable for comparative purposes, as it would be impossible to know whether the different samples of apples to be compared were in exactly the same state of development.

Accordingly mixed samples of the cut apple (Bramley Seedling) were taken for the comparison of mineral properties of apples grown on different soils, in the seasons 1923 and 1924. The apples were grown on the following soils: gravel, silt, Old Red Sandstone, chalk, fine silt, and two fen soils. Owing to the poor crop in 1924 samples of three of the varieties of apples used the year before were not obtained.

The probable error of the mean for determinations of total ash, potash, phosphate, and iron was found for the Canterbury apples grown on gravel soil by the analysis of thirty individual apples of the 1924 crop. Owing to the small amount of material in single apples, the weight of ash available for mineral analysis was only about 0.20–0.25 grm., so that only the total ash, potash, phosphate, and iron were determined in the series, but in interpreting the results it has been assumed that for the lime and magnesia the limits of significance are of the same order as for the phosphate, which had the highest probable error of those determined.

The figures for the probable errors are shown in Table VII.

TABLE VII.

*Probable Error of the Mean for Analyses of Bramley Seedling Apples from Canterbury (percentages).*

	Ash.		Potash ( $K_2O$ ).			Phosphate ( $P_2O_5$ ).			Iron ( $Fe_2O_3$ ).		
	A.	B.	A.	B.	C.	A.	B.	C.	A.	B.	C.
30 apples in sample	1.20	1.63	1.89	1.97	0.89	2.39	2.43	2.63	2.02	2.20	2.99
20 apples in sample	1.47	2.00	2.32	2.42	1.09	2.93	2.98	3.22	2.48	2.68	3.66

Figures in column A: errors on results referred to a basis of dry weight.

" " B: " " " fresh weight.

" " C: " " " total ash.

<sup>1</sup> Juice of Bramley Seedling apples from fen and silt soils expressed at different dates: the highest and lowest weights (grm.) of mineral constituents in 100 c.c. of juice and percentage of ash are as follows: *Fen* ash 0.224–0.207.  $K_2O$  0.126–0.103; 56.2%–49.7%.  $P_2O_5$  0.0161–0.115; 5.1%–7.8%.  $CaO$  0.0080–0.0060; 2.68–3.5%. *Silt* ash 0.300–0.268.  $K_2O$  0.1662–0.1484; 56.0–54.8%.  $P_2O_5$  0.0228–0.201; 7.9%–6.7%.  $CaO$  0.0096–0.0072; 3.4%–2.3%. Traces of copper and manganese, as well as excess of iron, were present, probably derived from the press and the freezing cylinders, in spite of their being silvered.

The probable error is very low, and as three times the probable error may be taken as significant it will be seen that all differences greater than 10 per cent. are significant and in some cases differences as low as 6 per cent. As the determination of probable error is a very tedious process, it was assumed that for the other Bramley Seedlings grown on different soils the probable error would be of the same order, for the probable error of determinations of nitrogen, acidity, and other properties is higher on the apples from Canterbury than from the other localities.

TABLE VIII. *Analyses of Bramley Seedling*

Figures in column A : percentage on the basis of dry weight.

„ „ B: „ „ „ fresh weight.

„ „ C: „ „ „ ash.

Soil and Locality.	Dry Wt.	Ash.		Potash ( $K_2O$ ).			Phosphate ( $P_2O_5$ ).			Iron ( $Fe_2O_3$ ).		
		A.	B.	A.	B.	C.	A.	B.	C.	A.	B.	C.
Alluvial gravel (Canterbury)	1923	11.26	1.998	0.2245	1.065	0.1198	53.4	0.216	0.0243	10.82	—	—
	1924	11.26	2.000	0.2250	1.071	0.1206	53.4	0.194	0.0218	9.69	0.0054	0.00061
Silt (Spalding)	1923	11.10	1.997	0.2200	1.098	0.1219	55.3	0.184	0.0204	9.27	—	—
	1924	11.60	1.955	0.2264	1.000	0.1160	51.2	0.234	0.0271	11.97	0.0040	0.00047
Old Red Sand- stone (Worcester)	1923	11.66	1.688	0.1964	0.896	0.1042	53.1	0.161	0.0188	9.57	—	—
	1924	11.66	1.688	0.1964	0.896	0.1042	53.1	0.161	0.0188	9.57	—	—
Chalk (Burwell)	1924	13.19	1.739	0.2290	0.845	0.1112	48.6	0.126	0.0166	7.25	—	—
Fen (Wisbech)	1923	10.59	1.845	0.1952	0.919	0.0971	49.7	0.146	0.0155	7.94	—	—
Fen (Burwell)	1923*	12.50	2.248	0.2807	1.120	0.1400	49.9	0.140	0.0175	6.23	—	—
	1924	11.72	1.580	0.1850	0.761	0.0893	48.3	0.136	0.0159	8.59	0.0033	0.00039
Fine Silt (Bristol)	1924	11.68	1.702	0.1970	0.853	0.0995	50.5	0.116	0.0135	6.85	0.0048	0.00056

\* Only 6 apples were analysed, and these were suffering from bitter pit.

The results of the analyses are shown in Table VIII. Before comparing the apples grown on different soils it is important to observe the extent of seasonal variation. This appears to be small when the two seasons' analyses of the apples grown on silt and gravel soils are compared. The total ash and potash differences between the 1923 and 1924 apples grown on gravel soil are under 1 per cent. and are negligible; for the apples grown on silt soil the total ash difference of 3 per cent. is non-significant, the variation in concentration of potash in the dry weight (column A) and in total ash (column C) is slightly above three times the probable error and is hence significant, but the variation in concentration of the potash in the fresh weight (column B) of twice the probable error is non-

significant. The seasonal differences of about 8 per cent. for the lime and magnesia in the apples grown on these two soils are also non-significant. The phosphate seasonal differences are the largest—for the apples grown on silt soil being six times the probable error, and for the apples grown on gravel soil three times the probable error. It thus appears from the analysis of these apples that, except for the phosphate, the seasonal variation in mineral content is small, especially in column B (percentage referred to fresh weight). For comparative purposes it is therefore better to

*Apples grown on Different Soils.<sup>1</sup>*

Mixed samples of 20 apples in 1923.  
 „ „ 30 apples in 1924.

<i>Lime (CaO).</i>			<i>Magnesia (MgO).</i>			<i>Nitrogen.</i>		<i>Ratios.</i>					
A.	B.	C.	A.	B.	C.	A.	B.	$K_2O/N_2$ .	$CaO/K_2O$ .	$P_2O_5/K_2O$ .	$P_2O_5/N_2$ .		
0.046	0.0052	2.31	0.033	0.0037	1.65	0.297	0.0334	3.59	0.043	0.203	0.728	} Class I.	
0.050	0.0056	2.49	0.030	0.0034	1.49	0.271	0.0305	3.94	0.047	0.181	0.713		
0.043	0.0048	2.18	0.041	0.0046	2.09	0.252	0.0297	4.36	0.039	0.168	0.764		
0.039	0.0045	1.99	0.042	0.0049	2.18	0.247	0.0287	4.04	0.039	0.234	0.846		
0.036	0.0035	1.78	0.034	0.0039	1.99	0.311	0.0362	2.88	0.034	0.180	0.518	} Class II.	
0.032	0.0042	1.83	0.038	0.0050	2.18	0.266	0.0351	3.17	0.038	0.149	0.472		
0.052	0.0055	2.87	0.035	0.0037	1.90	0.340	0.0359	2.74	0.057	0.160	0.438	} Class III.	
0.045	0.0056	1.99	0.044	0.0055	1.96	0.344	0.0430	3.26	0.040	0.125	0.407		
0.039	0.0046	2.49	0.031	0.0036	1.94	0.344†	0.0409†	2.18	0.052	0.178	0.388		
0.045	0.0053	2.69	0.031	0.0036	1.85	0.278	0.0325	3.07	0.053	0.136	0.417		

† Nitrogen estimated on a different sample.

consider chiefly the percentages referred to fresh weight. The small value for the seasonal variation is interesting as the seasons were very different, 1924 being an exceptionally wet season and bad for fruit growing. The apples from Burwell for the two years are not comparable owing to the 1923 sample containing only six apples all of which showed 'bitter pit'.

In Table VIII the apples are arranged as far as possible in descending order of total ash, potash, and phosphate content, and it will be seen that large and significant differences are caused by the nature of the soil. The apples may be divided into three classes: Class I, containing the apples

<sup>1</sup> The data for iron are all too high owing to minute contamination from the metal fittings of the grinder; the results are probably comparable among themselves.



grown on silt and gravel soils, with the highest values of ash, potash, and phosphate; Class III, containing the apples grown on the two fen soils and fine silt soil, with the lowest total ash, potash, and phosphate values. Class II contains the apples grown on the other two soils, Old Red Sandstone and chalk; it is not so well defined, but is intermediate in properties between the other two.

If Class I (apples from silt and gravel soils) and Class III (apples from two fen soils and fine silt soil) are compared, significant differences of 15 per cent., 20 per cent. are observed between their total ash values and their potash values, or about six or seven times the probable error, and the phosphate differences are from three to ten times the probable error; in each case Class I has the higher value. The lime and magnesia differences are not well defined, as significant differences are shown between the members of the same class. It appears that Class I tends to have a low, and Class III a high nitrogen value. Class II (apples grown on chalk and on Old Red Sandstone soils) resembles Class III in its percentages of total ash and potash referred to dry weight (column A), the values of which are 12 to 15 per cent. lower than in Class I, though for the apples grown on chalk soil the percentages of potash and total ash referred to fresh weight (column B) are similar to Class I and significantly higher than in Class III. The phosphate values of the apples grown on Old Red Sandstone are significantly higher than in Class III, and three times the probable error lower than for Class I for the apples grown on the chalk soils; the phosphate content is similar to Class III, and is only significantly higher than in the apples grown on fine silt soil in Class III. The nitrogen percentages for these apples are also intermediate between the two other classes.

According to Gardner, Bradford, and Hooker (7), there is a relation between the nitrogen and phosphorus for practically all tissues of the plant. Hence the ratio of the two in the apple should be characteristic for the different localities, and it will be seen that the phosphate-nitrogen ratios are highest for the apples in Class I, intermediate in Class II, and lowest in Class III. It is also noticeable that the seasonal variation is small. The potash-nitrogen ratio is also highest in Class I, but information as to the incidence of leaf-scorch (see below, apples on different stocks) in the orchards has not been obtained.

The marked difference between the three classes of apples is to be expected from the nature of the soils. Silt and gravel soils are somewhat similar; they are medium light soils and contain a larger percentage of fine sand and silt to clay particles than fen and fine silt soils, which are heavy soils, while chalk soils (from fen locality) are intermediate. The apples from the fen soils are softer in texture and do not keep so well as the apples from silt soils; these differences however may be connected with the

high moisture content of fen soils. The similarity between the members of the same class, especially Class I, grown on gravel and silt soils, is very noticeable; the differences in their analyses are all less than the seasonal variation, except between the lime and magnesia values for 1924, where the differences are about 20 per cent., the apples grown on gravel soil having the higher lime and lower magnesia values. The low magnesia value of the apples from Canterbury (alluvial gravel) is in keeping with the qualities of the soil, as according to Hall and Russell (9) the Thanet beds of Canterbury contain a very low percentage of magnesia. These apples keep better and are firmer in texture than those grown on fen soils. It is possible that the superiority of the apples grown on the gravel and silt soils may be partly due to their high percentage of phosphate and potash, which seems to be directly due to the qualities of the soils. According to Hall (8) silt soils usually contain more phosphate and potash than fen soils, and in the gravel soil the available phosphate is very high, though the total phosphate is low; it is the reverse with the potash, but the Canterbury orchards are manured with potash, which probably accounts for the high percentage of potash in such apples.

The apples in Class III grown on the two fen soils (Wisbech and Burwell) and on fine silt soil also resemble each other very closely,<sup>1</sup> especially in the concentration of minerals in the fresh weight (column B). The apples grown on fen soil at Wisbech and fine silt soil at Bristol resemble each other very closely, only the phosphate difference of 12 per cent. is significant, the other differences being less than 4 per cent. The mineral content of fen apples from Burwell is slightly lower than in the other two members of the same class, the total ash and potash values are 10 per cent. lower, and the lime percentage is 14 per cent. lower; however, the phosphate and magnesia differences from the apples grown on fen soil at Wisbech are less than 4 per cent. The apples on the two fen soils have the highest nitrogen percentages. The phosphate-nitrogen ratios for the apples grown on all these three soils are also of the same order.

The similarity of these apples is in keeping with the similarity in the types of the soils on which they were grown. Analyses of the soil from the actual orchards is available from Burwell (fen soils) and from Bristol (fine silt). It may be assumed that the mechanical analyses of the two fen soils (Wisbech and Burwell) are similar, and from the analyses Bristol (fine silt) and Burwell (fen) appear to be somewhat similar; their ratios of fine sand and silt to clay particles are of the same order (1.7 and 1.5), but of the two Bristol contains a larger percentage of fine silt particles. In accordance with the low potash and phosphate percentages in the apples from the fen and fine silt soils the available potash and phosphate in the soils is low, and the amount of organic material is higher than in silt soils.

<sup>1</sup> The results of the analysis of the apples suffering from bitter pit is neglected here.

The analysis of the Burwell chalk soil is also available, and it is interesting that, of the three analyses available, the percentages of phosphate referred to dry weight are in the same order as the percentages in the soil.

Concerning the analysis of the apples suffering from bitter pit from Burwell in 1923, the high ash compared with the value in 1924 is very noticeable. This difference is a real one, although in 1923 there were only six apples in the sample, so that the probable error for the percentage of ash referred to dry fresh weight was 3.6 per cent. ; when Fisher's method (7) for estimating the difference between two means was used, the value of  $t$  was found to be 3.2, showing that the difference was significant. This high value for the ash agrees with the analysis quoted by McAlpine (16). 'Pitted' and healthy apples from the same tree were taken for several varieties, and in each case the ash was considerably higher in the 'pitted' apples. Nitrogenous manuring is supposed to increase the susceptibility to bitter pit, and it is interesting that Burwell apples have the highest nitrogen values. It is also claimed that phosphate fertilizers are good as a preventative, and it will be seen that the percentage of phosphate in the apples suffering from bitter pit is very low, indicating that with the increase in the quantities of mineral constituents absorbed, a corresponding increase in the amount of phosphate was not available. This low value for the phosphate concentration in the ash in 1923 is 25 per cent. lower than in 1924, and may be said to be significant.

From these analyses of apples grown on different soils it appears that there is a very definite relation between the type of soil and the mineral analysis of the apples.

#### *Analysis of Apples grown on Different Stocks.*

Table IX shows the results of analyses of Lane's Prince Albert apples grown at the East Malling Research Station on stocks, Types I, II, IX, and X, which differ markedly from each other in their effect on the scion. The different qualities of the stocks have been described by Hatton (12, 13, 14); Type X is a very vigorous stock, late in maturing, and trees grown on it fruit later than on the other three types. It is used for standard and half-standard purposes. Type I, or Broadleaf English Paradise, is a vigorous stock late in maturing and slow in cropping; it is suitable for large permanent bushes of weak-growing varieties of apples. Type II, or Doucin, is a semi-dwarfing stock suitable for semi-dwarfing bushes or fillers; it is more precocious in cropping than Types I and X. Type IX, or Jaune de Metz, or Yellow Paradise, is a very dwarfing stock, suitable for cordons and for quickly fruiting new seedlings. It is very early maturing and fruits early and heavily.

The probable error of the mean of these Lane's Prince Albert apples was not determined, but it may be assumed that it is of the same order

as that of the Bramley Seedling apples; thus total ash and potash differences of more than 8 per cent., and phosphate and iron differences of more than 10 per cent., may be accepted as significant.

The 1924 apples were gathered before they were ripe, on September 12, owing to their being damaged by a severe hailstorm. They show a higher percentage of protein nitrogen than those of 1924; these determinations were kindly made by Miss Archbold. It is interesting to note that, in spite of the immaturity of the apples of 1924, the seasonal variation is small, as it was for the Bramley Seedling apples, and similarly that the least seasonal variation is shown in the concentration of minerals in the fresh weight (column B). For Type I the seasonal difference in column B for total ash is 4 per cent., potash 1 per cent., phosphate 4.5 per cent., and magnesia 0 per cent., all of which are non-significant. In column A (percentage referred to dry weight) the ash, potash, and magnesia differences of 20 per cent. to 15 per cent. are significant, but the phosphate difference remains non-significant, and the lime difference of 3 per cent. is also without significance. In Type IX the seasonal variation appears to be even less, as all the differences are non-significant.

It is interesting to discover whether apples grown on similar stocks are similar in mineral constitution; stocks I and X are both vigorous types, and on comparing the figures in Table IX for these two stocks, it will be seen that all their differences are non-significant, and are of the same order as the seasonal variation—about 4 per cent. However, Type II and Type IX are also similar, in that they are both dwarfing stocks, but their mineral properties are very different, Type II containing more, and Type IX less, total ash and potash than the other types. If the concentration of the minerals in the fresh weight (column B) is compared, significant differences between the types are shown. In Type II the total ash and phosphate figures are 10 per cent. higher, and in Type IX are 10 per cent. lower, than for the two vigorous Types I and X. The phosphate percentage in Type II is 15 per cent. higher than in the other three types, though on comparing the phosphate figures in column C (percentages referred to total ash), the two dwarfing stocks, Types II and IX, are shown to have the higher values, especially in 1924, when the difference between the figures for Type I and Type IX was 20 per cent. The seasonal variation in the lime content is large (probably due to the difference in the state of maturity), so that it is difficult to compare the differences between the stocks, but the two dwarfing types tend to have a lower value than the more vigorous stocks. The iron percentages (column B) in Types II and IX are also similar, and are about 25 per cent. higher than Type I. In column C (percentage referred to total ash) the very high percentage of iron in Type IX is noticeable; it is more than 30 per cent. higher than for Type II, which is 16 per cent. higher than Type I. The nitrogen content

of Type II is considerably higher than in the other types. The properties of the juice (1924) do not show any similarity between Types II and IX, except in the pH values, which are in order of the vigour of the stocks, the most vigorous having the highest hydrogen-ion concentration. The high value for potassium malate in the juice of Type II is in keeping with the high potash concentration found in the apple; Type II has the most acid, and Type IX the least acid juice.

It is possible that the difference in mineral constitution between Types II and IX may cause some of the difference in resistance observed between

TABLE IX. *Mineral Constitution of Lane's*

Mixed samples of 20 apples.

Stock.	Dry Wt.		Ash.		Potash ( $K_2O$ ).			Phosphate ( $P_2O_5$ ).			Iron ( $Fe_2O_3$ )		
	B.	A.	B.	A.	A.	B.	C.	A.	B.	C.	A.	B.	C.
Type I, 1923	12.96	1.398	0.1797	0.755	0.0976	54.3	0.19	0.024	13.4	—	—	—	—
1924	10.95	1.669	0.1827	0.897	0.0983	53.8	0.21	0.023	12.8	0.0042	0.00046	0.25	—
Type II, 1924	10.87	1.890	0.2098	1.017	0.1104	53.9	0.27	0.029	14.1	0.0055	0.00060	0.29	—
Type IX, 1923	11.50	1.388	0.1596	0.769	0.0883	52.2	0.20	0.023	14.2	—	—	—	—
1924	11.48	1.349	0.1546	0.708	0.0811	52.5	0.21	0.024	15.5	0.0054	0.00062	0.40	—
Type X, 1923	12.88	1.461	0.1880	0.790	0.1016	54.0	0.19	0.025	13.3	—	—	—	—

*Properties of the Juice, 1924*

Stock.	pH.	Acidity.	Density.
Type I	2.72	0.0995 N.	1.043
Type II	2.78	0.1029 N.	1.041
Type IX	2.83	0.0785 N.	1.043

these stocks otherwise somewhat similar. In the work done by Hatton and Grubb (11) on the incidence of leaf-scorch on the apples at East Malling it was found that trees on Type II had some scorch, on Type I less scorch, and on Types IX and X little or no scorch. The work of Wallace at Long Ashton (26) points to the fact that the incidence of leaf-scorch is due to a low potash-nitrogen ratio, plants being made less susceptible either by increasing the potash or decreasing the nitrogen in the culture solution. It has also been found that the incidence of scorch is connected with cropping; where there is less crop there is less leaf-scorch. Analyses made by Warren (25) show that there is less potash in the leaves when the yield is greatest, and this may cause the leaves to be more susceptible to scorch when the crop is large, unless the plant contains a low percentage of nitrogen. It will be seen (Table IX) that the potash in the apples on Type II is higher, and on Type IX is lower than the rest. Thus when

Type IX bears a large crop, it does not cause so heavy a drain on the potash resources of the tree as does Type II, and this, as well as the difference in nitrogen content, may in part explain the comparative immunity of Type IX from leaf-scorch, while Type II is susceptible. However, Types I and X, which are both slow in cropping and their potash differences from each other are insignificant, are slightly differently susceptible to leaf-scorch; this may be due to the nitrogen content, as Type I contains a larger percentage than Type X. In this connexion it is interesting that Type IX has been found by Massee (17) to be resistant to

*Prince Albert Apples on Different Stocks.*

Mixed samples of 20 apples. A, B, and C as in Table VIII.

Lime (CaO).			Magnesia (MgO).			Nitrogen.		Ratios.				
A.	B.	C.	A.	B.	C.	A.	B.	K <sub>2</sub> O/N <sub>2</sub> .	CaO/K <sub>2</sub> O.	P <sub>2</sub> O <sub>5</sub> /K <sub>2</sub> O.	P <sub>2</sub> O <sub>5</sub> /N <sub>2</sub> .	
0.032	0.0042	2.34	0.053	0.0068	3.78	0.405	0.0525	1.872	0.043	0.246	0.0456	
0.033	0.0036	1.97	0.062	0.0068	3.72	0.741	0.0812	1.211	0.037	0.34	0.0284	
0.029	0.0031	1.52	0.065	0.0071	3.47	1.000	1.0087	1.015	0.028	0.263	0.0455	
0.032	0.0037	2.32	0.050	0.0058	3.63	0.440	0.0506	1.811	0.042	0.260	0.0455	
0.028	0.0032	2.07	0.053	0.0061	3.94	0.685	0.0786	1.034	0.029	0.296	0.0305	
0.030	0.0039	2.08	—	—	—	0.386	0.0498	2.033	0.038	0.247	0.0501	

attacks of green apple aphis, and Staniland (22) has found the same type resistant to woolly aphis, while the other types were attacked.

SUMMARY.

Methods specially adapted for the determination of (1) the total ash, (2) potash, (3) lime, (4) magnesia, (5) phosphate, (6) iron, in apples are described.

Analyses were made of dried apple material, as analysis of juice did not give consistent results.

The six determinations mentioned above were made on Bramley Seedling apples grown on different soils in 1923 and 1924, and on Lane's Prince Albert apples grown on four East Malling stocks for the same seasons.

Separate analyses were made of thirty individual Bramley Seedling apples from one orchard at Canterbury in 1924. The errors of the mean result were found to be:—ash 1.63 per cent.; potash 1.97 per cent., phosphate 2.43 per cent., iron 2.20 per cent., for results referred to a basis of fresh weight, therefore, differences exceeding three times the probable error have been taken as significant.

Seasonal differences between analyses made in 1923 and 1924 were

found on the whole to be non-significant, especially in the concentration of the mineral substances in the fresh weight.

The analyses of Bramley Seedling apples grown on different soils showed significant differences; apples grown on gravel and silt soils were found to be similar, having high total ash, potash, and phosphate percentages, while apples grown on fen (from Wisbech and Burwell) and fine silt soils were characterized by low values for total ash, potash, and phosphate percentages. Good keeping qualities were found to be associated with high percentages of potash and phosphate.

Apples suffering from bitter pit showed high ash values and a low percentage of phosphate in the ash.

From the analyses of apples grown on different stocks, it appeared that, while apples grown on the two vigorous stocks, Types I and X, were similar in mineral constitution, the apples grown on the two dwarfing stocks, Types II and IX, showed significant differences, Type II having significantly higher percentages of total ash, potash, and phosphate than the other stocks, while Type IX had lower values for total ash and potash than the other stocks.

Some evidence supporting the theory as to the importance of the potash-nitrogen ratio in connexion with the incidence of leaf-scorch was obtained.

The writer wishes to thank Dr. H. Harwood, in whose laboratory the work has been carried out, for much helpful advice on methods of mineral analysis, also Professor V. H. Blackman, under whose direction the work has been undertaken, and Dr. D. Haynes, for their kind help and criticism.

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# On the Relation of Certain Soil Algae to some Soluble Carbon Compounds.

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With Plate VII and thirteen Figures in the Text.

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## PART I.

### PRELIMINARY STUDIES ON SOIL ALGAE.

#### (I) INTRODUCTION.

IN attributing to various groups of organisms the part that they play in the economy of nature, it would be generally agreed that the algae, by virtue of their possession of chlorophyll, must act under the stimulus of light as producers of organic substances and as storehouses of the energy which they derive from the sun, and that they attain their maximum

economic importance in the ocean, where the whole of the animal life is ultimately dependent on them for food. Recent research has shown, however, that certain of the lower algae may act, on the contrary, as consumers of many soluble organic substances, particularly the carbohydrates, and that a number of species may even break down complicated proteins by the secretion of enzymes. In these cases they must derive energy from that set free by the chemical decomposition of the compound consumed. It has also been found that, given suitable sources of carbon and nitrogen in their food-supply, certain species of algae are able to grow in the dark. The relation of the algae to light and to carbon-supply has been considered briefly by the writer in an earlier publication (Bristol, 3), where a bibliography is given of the most important papers on the subject. None of the forms dealt with in these papers are algae from soil, and a detailed consideration of them is therefore omitted from the present paper.

It appears from the current literature that different species of algae may vary considerably in their mode of nutrition, even though morphologically they may be very nearly related, and that the physiology of each organism must be studied separately before a coherent idea may be obtained of the economic function of the algae as a whole. Among the fungi and bacteria the idea of specialization in nutrition is now so well established that a specific relation between a fungus and its host is accepted without question, while the isolation of soil bacteria which can grow with phenol or naphthalene as their sole source of carbon (Gray, 7) causes comparatively little comment. It is possible that other relationships equally striking may be observed among algae hitherto assumed to be autotrophic.

These considerations are of extreme importance in regard to the algae of the soil; apart from them it would be difficult to conceive of the existence of algae within the soil in any other condition than a resting state, and it would inevitably follow that the mass of algae which appears when a small quantity of soil is placed in a flask containing a sterile solution of mineral salts<sup>1</sup> must arise by the germination of resting cells. The results of earlier experimental work (Esmarch, 9; Moore and Karrer, 12; Bristol, 3), though not conclusive, suggest that many of the algae within the top six inches of the soil are present, on the contrary, in a vegetative state, and their effect on the fertility of the soil thus becomes a question of great interest.

The methods of counting so commonly used for following the sequence of changes in other groups of soil organisms have been found (Bristol, 3) to have comparatively little value when applied to the study of the algae, on account of the gelatinous covering with which many of the soil species

<sup>1</sup> Esmarch (8, 9), Robbins (14), Petersen (18), Moore and Karrer (12), Bristol (1, 2).

are invested. By shaking an aqueous suspension of soil it is quite impossible, for example, to break up a *Nostoc* colony successfully, or to separate chlorelloid cells which have become entangled among a mass of *Phormidium* filaments; the results obtained by such methods therefore give but a rough idea of the extent and variability of the algal population from week to week. Hence it seemed best, in studying the soil algae, not to pursue this direct method of observation, but to develop the work along physiological lines by isolating the organisms from the soil and studying their activities under controlled conditions, with the hope of throwing some light on their relation to the fertility of the soil.

For this purpose it became necessary to obtain the organisms in pure culture,<sup>1</sup> so that their physiological reactions could be observed away from the disturbing influences of other organisms.

## (2) METHODS OF ISOLATION AND PURIFICATION OF SOIL ALGAE.

To separate a number of species of algae from one another is a comparatively easy task, but to free such unialgal cultures from other contaminating organisms, especially from bacteria, is an undertaking requiring a great deal of time and patience and a certain dexterity in manipulation. Of a number of special methods that have been described, that of Chodat (1909, pp. 36-43) is probably the most generally useful for the unicellular algae; it consists chiefly in growing the organisms in a very dilute solution of mineral salts (Detmer's solution<sup>2</sup> diluted to one-third its normal strength, made up with tap-water and containing 0.01 per cent. ferric chloride), the nutrient value of the solution being very low and the acidity so high that bacteria do not readily grow in it. The cultures are exposed to bright sunlight, which stimulates the growth of the algae but retards the development of the contaminating organisms. Successive subcultures are made in this way until the proportion of algae to contaminating organisms is very high; dilute suspensions of the alga are then made in sterile water and well shaken to separate the cells as far as possible from the contaminating organisms. Varying quantities of the suspension, calculated to give 1, 2, 4,.....64 colonies per flask, are then inoculated into flasks containing melted agar medium at 42° C. and well shaken up; the colonies are allowed to develop in bright sunlight within the solid agar. By this method the growing colonies are kept separate from one another, and the algal colonies can be cut out aseptically and subcultured on to a fresh medium. If this

<sup>1</sup> The writer was enabled, by means of a Travelling Fellowship from the Ministry of Agriculture, to spend three months in the laboratory of Professor R. Chodat at Geneva, studying under his supervision the methods which he has developed for this purpose. She would like to take this opportunity of expressing her appreciation of the interest which he took in her work and of thanking him for his helpful suggestions.

<sup>2</sup>  $\text{Ca}(\text{NO}_3)_2$ , 1 grm.;  $\text{KCl}$ , 0.25 grm.;  $\text{MgSO}_4$ , 0.25 grm.;  $\text{KH}_2\text{PO}_4$ , 0.25 grm.; water, 1 litre.

does not give the required result the first time, it is necessary to repeat the whole process again until it happens that an algal cell is embedded in the agar free from all contaminating organisms and therefore capable of producing a pure colony.

The method was adapted to the isolation of soil algae in two ways:

(a) Algae were obtained as separate colonies directly from the soil by inoculation of a soil suspension into a sterile agar medium. About 5 gm. of soil were shaken thoroughly with 5 c.c. of water to make a uniform suspension; ten drops of the suspension were added to 10 c.c. of diluted Detmer's solution and the whole well mixed. Further suspensions were made by adding 2, 4, 6.....18 drops of the second suspension to a number of tubes containing 10 c.c. of the same nutrient solution. The tubes were well shaken, and 6 drops of the suspension were transferred from each to a small Erlenmeyer flask containing melted mineral salts agar at 42° C. The medium was allowed to cool and then exposed to sunlight in a south window.

After a few days, colonies of fungi and bacteria were observed within the agar, but through the action of the light the growth of the fungi was greatly retarded and the bacterial colonies did not spread. The first signs of algal colonies were observed fourteen days after inoculation, but they were left to grow until, at the end of five weeks, they were about 1 mm. in diameter. Many of them, surrounded by a small amount of agar, were then cut out with a sterilized knife and transferred with a sterilized needle on to mineral salts agar and into diluted Detmer's solution to allow unimpeded growth. The colonies so isolated were all contaminated, and it was therefore necessary to proceed with the purification as described above.

(b) The algae were obtained in mixed culture by inoculating small quantities of soil into nutrient solutions, and were afterwards separated and purified. It was feared that inoculation into diluted Detmer's solution might give rise only to those algae that are essentially autotrophic in nutrition, and that the more typical soil forms which must be saprophytic might be unable to grow in so poor a medium, even in sunlight. Inoculations were therefore made into four different nutrient solutions, viz.:

- A. Detmer's mineral salts solution diluted to one-third, without iron, since there was likely to be sufficient iron in the inoculum for the growth of the algae.
- B. The same + 0.01 per cent. ferric chloride.
- C. The same + 0.01 per cent. ferric chloride + 1 per cent. glucose.
- D. The same + 0.01 per cent. ferric chloride + 1 per cent. glucose + 0.1 per cent. peptone.

Cultures in C and D were observed after three days to be teeming with bacteria and fungi, which continued to grow at such a rate, even in sunlight,

that the algae were unable to develop; both media were therefore discarded as unsuitable for the purpose.

The first signs of algal growth were observed in medium B on the seventh day as a green rim around the top of the liquid; the algae quickly spread over the whole surface and formed a stratum on the walls of the glass vessel within the liquid. Microscopic examination after five weeks showed that there were present green, yellow-green, and blue-green forms, but, owing to their juvenile state, it was not possible to identify them accurately. Algae appeared in medium A ten days later than in medium B, and the stratum was less extensive; it contained green and yellow-green forms, but blue-green algae were absent. The medium appeared therefore to be much less suitable for algal growth than B, and no further attempts were made to use it.

Five weeks after inoculation, representative portions of the algal stratum from a culture in medium B were placed in water on a sterile slide and broken up thoroughly by friction with a sterile cover-slip. The material was then transferred to a test-tube containing 10 c.c. of sterile water and shaken vigorously for ten minutes. The number of organisms per drop of suspension was counted under the microscope, and dilutions were made from it to give 1, 2, 4.....512 cells per drop. Single drops were transferred from each of these dilutions to small Erlenmeyer flasks containing melted mineral salts agar at 42° C. and thoroughly shaken. The cultures were then put in direct sunlight to grow. The stratum from a culture in medium A was similarly treated.

Three weeks later a number of algal colonies were cut out from the flasks and transferred aseptically to mineral salts agar or to Detmer's solution. A few, especially *Nostoc* colonies, were placed on porous porcelain tiles or silica jelly impregnated with Detmer's solution. Purification of the cultures was then attempted as previously described.

This second method of isolation of the algae from the soil has certain disadvantages compared with the first method: it takes a much longer time, and it provides an opportunity for the more rapidly growing forms to predominate in the mixed culture, so that other less rapidly growing species may be quite absent from the final dilutions. This is what actually happened in the cultures in medium A; of thirteen colonies cut out from three culture flasks twelve were identical.

When an algal culture seemed to have been freed from contaminating organisms it was transferred to a number of media more suitable for fungal and bacterial growth in order to establish its purity. Stock cultures are kept growing on media containing glucose, so that bacterial contaminations may be readily recognized. Experience has shown that Erlenmeyer flasks of 70 c.c. capacity, containing 20-30 c.c. of medium, are the most suitable culture vessels for algae.

Up to the present time pure cultures have been obtained of nine or ten unicellular green algae, one or two yellow-green forms, and one blue-green species, a small species of *Phormidium* with tortuous filaments. A number of others are in course of purification, including the commonest soil diatom, *Hantzschia amphioxys*; but so far all attempts to purify *Nostoc* colonies have been quite unsuccessful.

### (3) GROWTH ON VARIOUS CULTURE MEDIA.

Stab subcultures of the pure algae were made on a number of different agar media, including a variant<sup>1</sup> of that recommended by Moore (11) and by Schramm (15), and the modifications of it employed by Wann (18). These cultures demonstrated a number of conspicuous differences in the physiological reactions of the different forms. It was observed that the addition of 1 per cent. glucose to any mineral salts medium increased to an enormous extent the growth of all the species except two, a small species of *Chlorococcum* (No. 3), and the *Phormidium* sp. (No. 4). In the case of *Phormidium* sp., the inoculum rapidly lost colour and died on any medium containing a sugar, while in the case of *Chlorococcum* sp. there was simply no acceleration of growth. This suggests that these two species are autotrophic in nutrition and may grow only on the surface of the soil, whereas the other species are capable of absorbing certain soluble organic substances and may have a much more extended distribution.

Some of the species were further subcultured on to a series of agar media consisting of mineral salts + 1 per cent. of various organic compounds. Very striking differences in the behaviour of the species appeared on these media, for though in almost all cases glucose produced a maximum amount of growth, the order of availability of the other compounds differed for each species. Culture No. 16, for example, a species of *Cystococcus*, grows equally well, if not better, with mannite than with glucose, whereas Culture No. 11 grows less well with mannite than with mineral salts alone.

It was soon realized, however, that the method of inoculating these cultures, i. e. by stabbing with a platinum needle, did not ensure the introduction of a standardized inoculum, and that the age and condition of the parent culture had a marked effect on the growth of the daughter cultures, while the intensity of illumination, the temperature of the room, and the season of the year were also important factors in determining their growth. Chodat's separation of nearly related species on the basis of the size of the

<sup>1</sup> The prescription for this medium was taken from Wann's paper; in the absence of any specification to the contrary it was assumed that 'MgSO<sub>4</sub> 0.2 gm.' referred to the anhydrous salt. In making up the medium 0.45 gm. of MgSO<sub>4</sub> · 7H<sub>2</sub>O was therefore used to give an equivalent amount of the anhydrous salt. On later reference to Schramm's paper it was found that 0.2 gm. of the hydrated salt should have been used. As the medium had been giving entirely satisfactory results, the alteration was adhered to in order to obtain continuity of method.

colonies produced in stab cultures on different agar media (Chodat, 1913) thus appears somewhat unreliable and needs further investigation, or at least more accurate specification.

Attempts were made to obtain reliable comparative results by inoculating the culture flasks with equal volumes of a well-shaken aqueous suspension of the alga to be investigated. This method gave much more dependable information about the relative amounts of growth of a given species on a series of media, but could less satisfactorily be applied in a comparison of the relative activities of two different species, on account of the difficulty of inoculating with equal amounts of the two organisms. The difference in size of the cells of two species has been found to render the number of cells an unreliable criterion of the size of the inoculum. A further difficulty in comparison arises from the fact that the liquid inoculum spreads unequally over the surface of the agar in the different flasks, and the algal cells may not be uniformly distributed.

The results of one such experiment are illustrated in Pl. VII. A series of media was made up having as a common basis the following modification of Moore's solution, recommended by Wann :

$\text{Ca}(\text{NO}_3)_2, 4\text{H}_2\text{O}$	1.475 grm.
$\text{MgSO}_4, 7\text{H}_2\text{O}$	0.45 grm.
$\text{K}_2\text{HPO}_4$	0.2 grm.
$\text{CaCl}_2$	0.1 grm.
$\text{FeSO}_4$	a trace.
Distilled water	1,000 c.c.

They were solidified with 1.5 per cent. agar, and contained respectively 1 per cent. of each of the following naturally occurring soluble carbon compounds :

(1) control	no carbon compound.
(2) mannite	alcoholic derivative of a hexose.
(3) glycerine	triose.
(4) xylose	} pentoses.
(5) arabinose	
(6) fructose	
(7) glucose	} hexoses.
(8) galactose	
(9) lactose	
(10) maltose	} disaccharoses.
(11) saccharose	

35 c.c. portions of the eleven media were measured out into small Erlenmeyer flasks of approximately equal capacity, and sterilized by steaming or by autoclaving as was most suitable for the sugar present. Sterile suspensions of pure cultures of two species of algae, *Scenedesmus costulatus*, Chod., var. *chlorelloides*, nov. var. (No. 11), and *Cystococcus* sp. (No. 16), were well shaken, and 0.25 c.c. portions of each suspension transferred with sterile



pipettes to four flasks of each medium and left to grow. It will be seen from the photograph that at the end of two months the cultures were strikingly different. *Scenedesmus* sp. (Pl. VII, series A) grew most rapidly on the glucose medium (7) and after some weeks began to lose its deep green colour and to become yellowish, finally assuming a cinnamon-red colour on the surface of the agar, though it remained green between the medium and the wall of the flask. It grew slightly less well at first on maltose, but at the time of photographing there was very little to choose between the two sets of cultures, except that those on maltose were still deep green in colour.

The most conspicuous feature of the cultures of series B, *Cystococcus*, sp., was the very luxuriant growth attained on four of the media; the actual bulk of alga was very much greater on mannite (2), glucose (7), fructose (8), and saccharose (11) than in any culture of series A, though the volume of the individual cells was not half as great as in the other species.

The relative amounts of growth in the two series of cultures are compared below, the sugars being placed in descending order of growth:

	No. 11.	No. 16.
<i>Degree of Growth.</i>	<i>Scenedesmus</i> sp.	<i>Cystococcus</i> sp.
Luxuriant to very good	glucose maltose galactose saccharose	mannite glucose fructose saccharose
Fairly good	— —	galactose maltose
Moderate	lactose glycerine fructose control	— — — —
Slight	mannite arabinose — — —	glycerine control lactose arabinose xylose
No growth	xylose	—

It appears therefore from these cultures that the two species can absorb not only glucose but also a number of other organic compounds in varying degrees. It is interesting to note that *Cystococcus* sp. grows most luxuriantly on two media, mannite and fructose, on which the *Scenedesmus* species grows feebly, and that it can grow slowly on xylose, which is found completely to inhibit the growth of the second species in the conditions of these experiments. On the other hand, the *Scenedesmus* species grows extremely well on maltose and galactose, which are much less useful to the *Cystococcus* than are several other substances.

It was thought that a quantitative estimation of the difference in growth of unicellular algae on various media might be made by using the media in liquid form, inoculating equal volumes of the fluid with equal quantities of

a well-shaken suspension of the cells, and counting the number of cells in each culture at the end of a given period of time. The method was tested by using a series of mineral salts solutions to which were added different concentrations of glucose and sucrose, as follows: M/100, M/50, M/30, M/20, and M/10 glucose, and M/200, M/100.....M/10 sucrose. Control media without sugar were used for comparison, the purpose of the experiment being to ascertain what concentration of sugar would produce the best results. Four species of algae were inoculated in triplicate into each concentration of sugar, viz. No. 2 (*Chlorella* sp.) and No. 7 (*Pleurococcus* sp.), in Moore's mineral salts solution, and No. 11 (*Scenedesmus costulatus*, var. *chlorelloides*) and No. 16 (*Cystococcus* sp.) in Wann's calcium nitrate modification of it.<sup>1</sup>

At the end of twenty-five days the number of cells per culture was counted in a Bürker blood-counting chamber, except in the case of species No. 16, of which the algal cells had become embedded in a continuous gelatinous stratum that could not be broken up satisfactorily. It was observed during the course of the experiment that the cultures in glucose of all four species grew more rapidly than those of the same species in the corresponding concentration of saccharose, and that the rate of development of each organism in the two sugars appeared, by the colour of the culture, to be proportional to the concentration of the sugar. It was therefore surprising to find, on counting the organisms, that the increments in number of cells were insignificant with increasing concentrations of sugar, except in the media with M/20 and M/10 saccharose; the cells, on the other hand, were very much larger in the media with a high concentration of sugar.

The actual figures, averages of three cultures, are given in Table I for the single species, No. 2, in the two series of media; separate controls were made for each sugar, the glucose cultures being counted four days later than the saccharose cultures.

TABLE I.

Concentration of Sugar per litre.	Number of Cells per c.mm. of Medium.	
	Saccharose.	Glucose.
No sugar (control)	2,400	4,000
200/M	4,900	—
100/M	3,300*	20,000*
50/M	4,700	25,900
30/M	4,700	25,900
20/M	7,000	26,100
10/M	8,500	10,700

\* Two cultures only.

<sup>1</sup> These four species were the subjects of an inquiry into the ability of green algae to fix atmospheric nitrogen (Bristol and Page, 4). The apparently arbitrary selection of solutions above was the outcome of observations made during the course of that investigation.

The fall in numbers in the M/10 glucose medium was obviously due to a staling of the medium, since many of the cells had assumed the appearance of resting cells containing an orange pigment, and a considerable number of small cells had begun to disintegrate.

It was concluded from the above experiment that, judging from the number, the size, and the general appearance of the cells, the medium containing M/20 glucose (i. e. 9 grm. per litre) had on the whole given the best growth of the four algae, and that 1 per cent. would be a useful concentration of sugar to use in algal media.

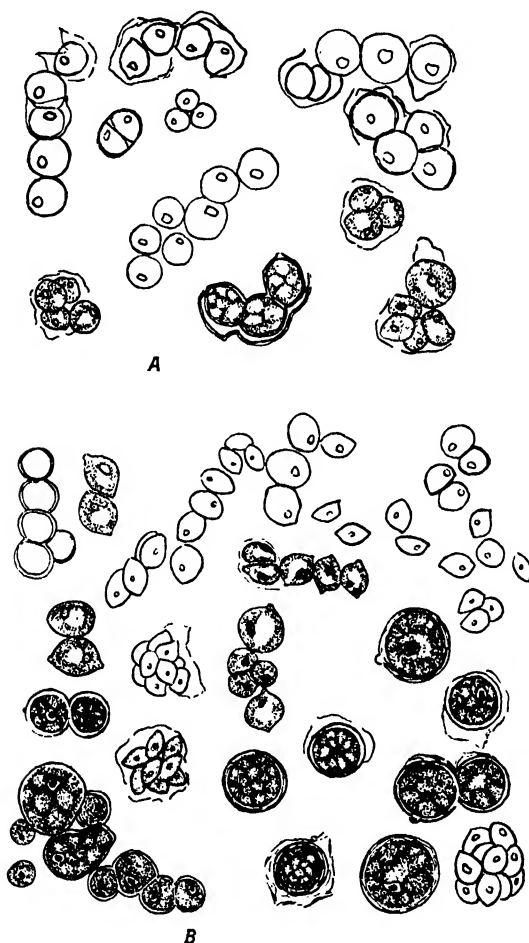
It was further concluded that much more reliable information could be obtained by taking daily measurements of the number and size of the cells from the time of inoculation of the cultures, and thence calculating the bulk of algal material, since this might give a better measure of the growth of the organism. This method would be suitable only for those organisms which, by shaking the medium, could be brought into a uniform suspension and counted with reasonable accuracy; but since a large proportion of the soil species are algae of this type the method could be applied fairly widely among them.

The organism selected for a preliminary trial of the method was No. 11, *Scenedesmus costulatus*, Chod., var. *chlorelloides*, nov. var., which, on account of its size and almost spherical form in liquid culture and of the very slight development of mucilaginous investment, lent itself most easily to the method.

Considerable difficulty has been experienced in naming this organism on account of its very indeterminate character, but constant observation during three years leaves little doubt that it is more nearly allied to Chodat's *Scenedesmus costulatus* (Chodat, 5, p. 102; 6, p. 38) than to any other described alga, differing from it chiefly in the much greater frequency with which it assumes the chlorelloid form. Casual observation might lead one to suppose that the organism is a species of *Chorella*, since in the majority of media, especially liquid media, coenobia are very rarely observed and the cells assume a spherical or subspherical form, multiplying by the formation of 4, 8, 16, or 32 autospores, which are frequently spherical in shape and are set free by rupture of the mother-cell-wall. On solid media, however, particularly on mineral salts agar and on the same with 1 per cent. glucose, coenobia can occasionally be seen, as illustrated in Text-fig. 1, and a good many cells assume the more elongated and fusiform shape so often associated with species of *Scenedesmus*. The walls sometimes exhibit thickenings, and on glucose media the cells periodically enter into a resting stage with a thick wall, often unequally thickened, a cinnamon-red pigment, and dense reserves of starch and oil. This causes the colony to lose its green colour and to assume a cinnamon<sup>1</sup> to whitish colour, beginning at the centre and leaving a green rim round the edge for a long period. After a period of rest the cells germinate again, and

<sup>1</sup> Ochraceous salmon xv, 13'b, to pinkish cinnamon xxix, 15'b, in Ridgeway's Colour Standards and Colour Nomenclature, 1912.

the whole colony gradually reverts to its original green colour,<sup>1</sup> often beginning at the middle. Smith (16) has relegated the species *Scenedesmus costulatus*, Chodat, to the class of 'doubtful or imperfectly described species', but the resemblance between Chodat's species and culture No. 11 is so considerable that, in the opinion of the writer,



TEXT-FIG. 1. *Scenedesmus costulatus*, Chod., var. *chlorelloides*, nov. var. A. From culture on mineral salts agar. B. From culture on glucose agar.  $\times 550$  approx.

Chodat's organism should certainly stand as a definite species, albeit one that is somewhat anomalous in character. Culture No. 11 deviates from Chodat's species in the colour assumed by the colony on glucose, and by the fact that the green colour is often completely regenerated

<sup>1</sup> Dark cress green xxxi, 29"m, to dark dull yellow green xxxii, 31"m, in Ridgeway's Colour Standards.

at a later stage; its growth on lactose appears to compare unfavourably with Chodat's species, and the cells never attain the size of those of the typical form. Young cells are  $5-6\mu$  in diameter, and they generally increase in size to  $10-12\mu$  before the autospores are once more formed. On old glucose media and on certain other sugars resting cells may occasionally attain a diameter of  $15-16\mu$ , but this is very exceptional. It liquefies a gelatine medium containing 1 per cent. glucose completely but very slowly: unliquefied portions of gelatine have not been observed in old cultures, as described by Chodat for *Scenedesmus costulatus*. But above all the chlorelloid form predominates in most cultures. Hence it seems best to regard it as a new variety of Chodat's species, possibly due to its different habitat, under the name var. *chlorelloides*.

When grown in liquid media the cells of this species are found often to hang together in clumps of four, eight, or even sixteen, as a result of the incomplete dissolution of the mother-cell wall, but these clumps readily break up if the liquid is shaken vigorously, and it is possible to get a uniform suspension of the cells. The number of cells per unit volume of medium can then be counted in a Bürker counting-chamber and their volume recorded by mounting a drop of the suspension on a microscope slide and making camera lucida drawings of fifty consecutive cells.

According to Student (17) the experimental error of the counts of such a uniform suspension of cells is equal to the square root of the number of organisms counted, and therefore, during the first few days of the experiments, a sufficient number of drops was counted from each culture to reduce the error to about 10 per cent. In later stages two drops only were counted, unless the numbers in the two counts differed by more than the sum of their square roots, when the average was taken of four counts.

A measure of the diameter of each cell drawn was taken to the nearest half  $\mu$  in two directions at right angles. These lengths were found to be identical in most cases, hence it was assumed that the cells in liquid media are approximately spherical in shape, and that the volume of each can be taken to be  $\frac{4}{3}\pi r^3$ , where  $r$  is half the diameter which can be measured directly. For the few cells that were obviously not spherical the approximate volume was assumed to be  $\frac{4}{3}\pi \left( \frac{\text{diam. 1} + \text{diam. 2}}{4} \right)^3$ . The difference

in length between the diameters has rarely been observed to exceed  $1\mu$ . Since the volume of a sphere varies as the cube of the radius, it was necessary to calculate the value of  $r^3$  for each cell and find the average ( $R^3$ ) for the fifty cells: the bulk of alga per unit volume of medium was then calculated from the formula  $\frac{4}{3}\pi R^3 n$ , where  $n$  is the number of cells per unit volume.

From a number of preliminary experiments in a glucose medium it was found that the average size of the cells varied conspicuously from day

to day, and that the number of cells per unit volume of medium increased in a most irregular manner. The calculated values of the bulk, on the other hand, were found to lie on a continuous curve within the limits of experimental error; and the logarithms of such values, when plotted against time, were seen to lie reasonably close to a straight line for a limited period of growth, after which the slope of the line became progressively less steep (cf. Fig. 4). That is to say, in pure culture, in the medium under consideration, the organism increased in bulk at an approximately uniform rate for a limited period of time, after which the rate of growth progressively decreased.

Considerable deviations of the logarithms from the straight line were found to be produced by variations in external conditions of temperature light intensity, and further experiments were therefore carried out in order to determine the best method for conducting such investigations under uniform conditions. The period of uniform growth rate was found to last in different experiments from eight to ten days, the length of the period being partly determined by the size of the inoculum. Direct information on this point is not yet available, but there is evidence from the data that are gradually accumulating that the growth rate begins to fall off when the bulk of alga per unit volume reaches a certain concentration. The length of time taken to reach this concentration depends directly on the size of the inoculum, but other factors appear to be involved, such as the age and condition of the parent culture, the temperature, the degree of aeration, and the intensity of illumination. These factors were of less interest to the writer than was the relation of the rate of growth to the supply of soluble organic food. Their importance was recognized, however, and any disturbing effects they might be likely to produce were avoided to a considerable extent by keeping such conditions as uniform as possible; their further elucidation was set aside until the more pertinent problem has been investigated.

## PART II.

ON THE RATE OF GROWTH OF *SCENEDESMUS COSTULATUS*, CHOD., VAR. *CHLORELLOIDES*, NOV. VAR., IN PURE CULTURE, IN A NUTRIENT SOLUTION CONTAINING VARIOUS SOLUBLE ORGANIC COMPOUNDS.

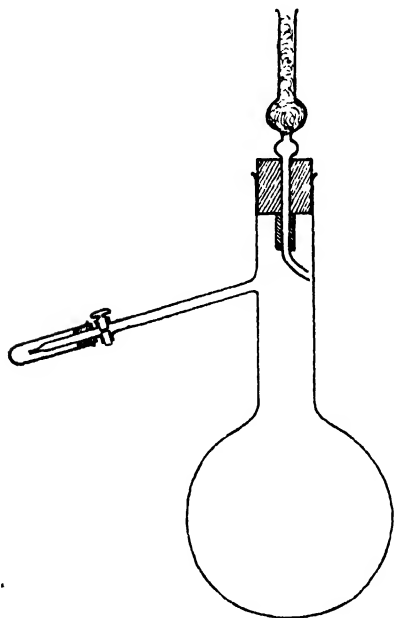
### A. EXPERIMENTAL METHOD.

The experimental method adopted for this investigation was gradually developed from experience gained in the preliminary experiments described above. A special method of sampling was devised in order to minimize the risk of contamination of the cultures during the course of the experiment, and as far as possible external conditions were kept uniform.

(a) *The Culture Vessel.*

The form of the culture vessel as finally approved for most of the experiments is illustrated in Text-fig. 2.

Distillation flasks of about 500 c.c. capacity were selected of as uniform a size and shape as possible, and, after being thoroughly cleaned with a saturated solution of potassium dichromate in 50 per cent. sulphuric



TEXT-FIG. 2. Drawing of culture vessel :  
for description see text.

acid, were washed carefully with distilled water and steamed for several hours. The neck of each flask was fitted with a one-holed rubber stopper through which passed the stem of a double-bulbed calcium chloride tube filled to the base of the upper bulb with cotton-wool. To the projecting end of the stem was attached, by means of pressure tubing, a short length of glass tubing<sup>1</sup> bent at such an angle that it almost touched the neck of the flask on the side opposite to the side tube. In this way the splashing of the cotton-wool plug during the shaking of the cultures was prevented. A glass tap was fused on to the end of the side tube<sup>2</sup> to facilitate the taking of samples from the culture, and the free end of the delivery tube was passed through a cork which fitted a small test-tube.

The liquid in the flask was well shaken to secure a uniform suspension of the algal cells and the flask then tilted so that the side tube became full. The bent tube attached to the end of the calcium chloride tube prevented the running back of the liquid into the cotton-wool plug when this was done. Holding the side tube vertically downwards, the tap was opened and the sample of culture fluid ran out into a prepared specimen tube.

When the mouth of the delivery tube was drawn out to a fairly fine point, the side tube did not completely empty itself, but remained closed to the air on account of a small drop of culture fluid that invariably remained in the mouth of the tube. The tap was closed with this drop still in position, and the mouth of the tube was dipped into a small test-tube, full

<sup>1</sup> In experiments requiring constant aeration of the medium a special form of apparatus was used as described in section A (i).

<sup>2</sup> In later experiments, owing to a number of breakages during sterilization, this connexion was made with pressure tubing bound on firmly with tape and rendered perfectly air-tight with melted paraffin wax. This method, though less convenient, has been found to work quite well.

of absolute alcohol, which fitted the cork on the delivery tube. The drop of culture fluid immediately flowed out into the alcohol on account of its greater density, and its place was taken by alcohol. The tap was again opened and the alcohol forced back up the delivery tube to within a few millimetres of the tap, which was then closed, and the tube of alcohol was secured in position by means of the cork. In taking subsequent samples it was necessary to wash out the delivery tube with one side tubeful of culture fluid, which was discarded, and then take a second sample on which to make the daily observations.

By adopting this device it became possible to open a pure culture flask any number of times with very slight risk of its becoming contaminated. The risk was further minimized by binding the outside of the tap with cotton-wool to prevent the entrance of organisms through the film of vaseline on the joint.

250 c.c. of the medium to be tested were placed in the culture flask, which was plugged with cotton-wool, and sterilized in a manner appropriate to the sugar contained in it; the tap was opened to prevent the collection of condensed moisture in the side tube, the end of which was covered with a thick pad of cotton-wool and wrapped in paper. The rubber stopper with its attached tubes was wrapped in paper and autoclaved separately.

#### (b) *Inoculation.*

Stock cultures of the organisms were grown on agar media, since this has been found to be the most satisfactory way of keeping them uncontaminated; and in the investigation under consideration inoculations were made, except where otherwise stated, only from those cultures grown on mineral salts agar without any sugar. As far as possible cultures were selected that were about ten weeks old, but owing to little-understood variations in the growth of subcultures this was occasionally impossible, and it was necessary to use cultures as old as four months. The colonies were transferred with a sterile glass spoon from the mother culture to a small sterilized Erlenmeyer flask containing 10 c.c. of distilled water and provided with a well-fitting cork and a strong paper cap extending well down the neck of the flask to prevent the collection of dust on the rim and the consequent possible washing in of contaminating organisms at later stages in the manipulations. The suspension was vigorously shaken for at least ten minutes, and the number of cells in two drops was then counted. By means of sterilized graduated 1 c.c. pipettes a sufficient quantity of the counted suspension was then transferred to the sterilized culture flasks to give a standard number of cells per unit volume of culture fluid, viz.  $1.3^1$

<sup>1</sup> The selection of this number was determined by the fact that, in the first experiment of this series, parallel glucose cultures inoculated with 1 c.c. of a suspension were each found to contain this number of cells.



cells per cubic millimetre, and the volume was recorded by making drawings of fifty cells in a drop of the original suspension. In this way cultures made at the same time would have approximately equal inocula, while those made at different times would vary according to the size of the cells of the mother culture.

After inoculation, the cotton-wool plugs were taken quickly from the culture flasks and replaced by the special sterilized stoppers freshly removed from their wrappings. These were bound into position with tape and rendered air-tight with melted paraffin wax. The taps on the side tubes were closed before the cotton-wool pads were removed, and the tubes of alcohol quickly put in position.

(c) *Temperature.*

After inoculation, the culture flasks were placed up to their necks in a water bath at a constant temperature of  $24.6^{\circ}\text{C}$ . for the earlier part of the investigation, the extreme variation about the mean being not more than  $0.05^{\circ}\text{C}$ . Later, the bath was moved to a room where the illumination could be regulated, and in so doing some slight alteration was made in the adjustment of the thermo-regulator which resulted in a constant temperature of  $24.4^{\circ}\text{C}$ .

(d) *Illumination.*

During the earlier part of the investigation facilities were not available for carrying out these experiments satisfactorily under conditions of constant light or of complete darkness. The water bath was therefore set up in the laboratory as far away from the windows as possible and out of the direct line of the sun's rays, so that the variation in intensity of the light should be reduced as much as possible. The bath was covered during those times when the electric light was burning in the room. Later, when a dark room became available for use, the cultures were grown with constant illumination from a single 60-watt gas-filled electric lamp at a distance of one foot from the surface of the liquid.

(e) *Shaking.*

The cultures were shaken by hand twice daily, at 9 a.m. and at 5.30 p.m., to ensure a constant supply of dissolved oxygen in the culture fluid and to break up the clumps of cells produced by the formation of autospores. This is an essential feature of the method, since a uniform suspension of single cells is absolutely necessary if the daily sample drawn from the flask is to be regarded as representative of the whole culture.

(f) *The Media.*

The mineral salts solution which formed the basis of all the media has already been described (p. 155). When first made up it is strongly alkaline,

owing to the reaction of the alkaline potassium phosphate (pH 8.9 in the salt used). The ferrous sulphate solution added reduces the alkalinity to some extent, and the medium is found to be extremely sensitive to the action of carbon dioxide from the air; so that if allowed to stand for twenty-four hours in the laboratory with the neck of the flask loosely plugged with cotton-wool the solution is found to become practically neutral in reaction (pH = 6.95). No attempt was made, therefore, to adjust the reaction of the medium to a definite level, but the plugged culture flasks were placed for twenty-four hours in the water bath after sterilization, to adjust themselves to the neutral point and to acquire the requisite temperature before inoculation.

To this basal solution was added 1 per cent. of the soluble organic compounds to be compared, equal weights of the compounds selected giving approximately equal numbers of carbon atoms per litre of medium, allowance being made for water of crystallization.

#### B. GROWTH IN A MEDIUM CONTAINING 1 PER CENT. GLUCOSE.

About twenty-five cultures of the organism have been studied in this medium, and it has been found that the more carefully are the external conditions kept uniform and the measurements made, the more nearly do the logarithmic values of the bulk fall along a straight line during the first nine or ten days of the growth of the culture. It was thought from some of the earlier experiments that this logarithmic period of growth was preceded by a short lag period, such as has been described by a number of workers for other organisms, but later experience has proved that such apparent lag periods were probably due either to faulty technique or to the use of an old mother culture containing a proportion of the cells in the resting condition.

The experimental error of the counts at the beginning of the culture is much higher when the cells are few than later in the experiment, necessitating the counting of a large number of drops in the early stages; and the drawing of fifty cells was so laborious that the number was reduced in the earlier cultures to twenty, with a corresponding increase in the experimental error. In later experiments the labour of drawing was greatly lessened by completely throwing the cells out of suspension in an electrically driven centrifuge and pouring off the greater part of the supernatant liquid. The deposit was suspended uniformly in the much smaller volume of liquid, making it easily possible to draw the requisite number of cells. From the time that this method was adopted no lag period has been observed in any of the cultures in a glucose medium.

(i) *The Behaviour of Parallel Cultures.*

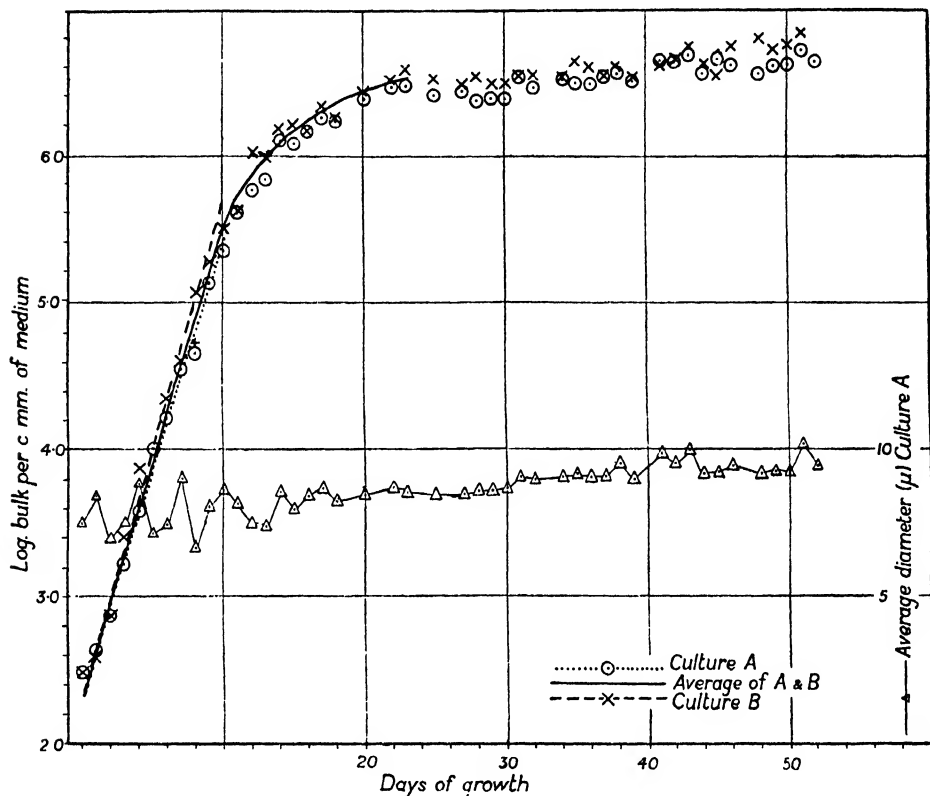
Some of the earliest experiments demonstrated the fact that, although the growth rate of the organism was approximately uniform in any given culture, yet cultures made at different times from different mother cultures did not necessarily grow at the same rate even in the same medium. Two parallel cultures in the glucose medium were therefore set up and inoculated with equal quantities (1 c.c.) of a well-shaken suspension of the organism, in order to ascertain to what extent the rate of growth would vary in cultures of the same origin kept under exactly the same conditions of light, and temperature, and grown in the same medium. Daily measurements were taken of the growth in each culture until the liquid was completely used up. The results of this experiment, both observed and calculated, are given for the two cultures in Table II.

An examination of the figures in the table brings out a number of interesting points. The size of the cells fluctuates in both cultures in the same orderly manner for the first eleven days of the experiment; the great majority of the cells of the mother culture were observed to be small; these took two days to increase to the size suitable for the production of autospores, and a large number of them then divided almost simultaneously into four daughter-cells, resulting in a considerable increase in numbers and a sudden drop in the average size of the cells after two days. It is seen from columns (4) and (9) that the remaining cells divided during the next two days, but the proportion of young cells was not sufficiently great to prevent a steady increase in the average size of the cells. A sudden drop in the size of the cells, accompanied by a considerable increase in number after five, eight, and eleven days respectively, marks further simultaneous divisions of a large number of the cells, and it seems safe to conclude that three days is the normal life of a cell of this species, as an individual, in the conditions of the earlier part of this experiment. After eleven days' growth the cells of the two cultures ceased to behave in a rhythmical manner in regard both to size and to number. This is extremely interesting because it is precisely at this point that the rate of growth, as indicated by plotting the logarithmic values of the bulk against time (Text-fig. 3), began to fall off in both cultures.

It was also observed in these cultures that after the growth rate had begun to fall off, there was a considerable increase in the proportion of cells dividing into eight autospores. This point was investigated carefully by mounting a drop of the suspension on a microscope slide on a number of different days, and making camera lucida drawings of all the cells that were in process of division, or had just finished dividing; within the outline of each cell was recorded the number of autospores formed. The percentages of the dividing cells, producing four and eight or sixteen autospores

respectively, were so strikingly different during the two periods of growth, that similar observations were made on a number of other cultures; the results of these observations are summarized in Table III.

The first six observations were made while the cultures were still growing at a uniform rate, and their agreement with one another is very



TEXT-FIG. 3. Diagram showing rate of growth of *Scenedesmus* sp. in two parallel cultures in a liquid medium containing mineral salts + 1 per cent. glucose: for explanation see text.

striking. Taking the six observations together, it was found that 379 of the 473 cells drawn (i. e. 80 per cent.) had divided into four, the rest into eight or sixteen, and that the proportions for the separate observations were very close to this figure. Similarly, the nine observations made after the growth rate had begun to fall off indicate that at this period the number of cells producing eight autospores was approximately equal to the number producing four. The drawings showed, further, that the modal value of the size of the dividing cells increased after the growth rate had begun to fall.

TABLE II. *Parallel Cultures in Liquid*

<i>Day of Expt.</i>	<i>Average Diameter. μ.</i>	<i>Culture A.</i>			
		<i>R<sup>2</sup>. μ<sup>2</sup>.</i>	<i>No. of Cells per c.mm.</i>	<i>Bulk per c.mm. μ<sup>3</sup>.</i>	<i>Log. Bulk.</i>
(1)	(2)	(3)	(4)	(5)	(6)
0	7.48	55.06	1.3	308	2.4879
1	8.4	77.73	1.3	434	2.6377
2	6.98	53.9	3.3	740	2.8693
3	7.55	59.41	6.7	1,060	3.2199
4	8.9	97.11	9.3	3,800	3.5794
5	7.09	51.09	47	10,000	4.0026
6	7.43	65.85	58.6	16,200	4.2087
7	9.03	106.56	77.8	34,700	4.5406
8	6.6	41.29	257	44,500	4.6493
9	8.05	70.07	462	135,700	5.1325
10	8.63	89.19	604	225,600	5.3534
11	8.13	79.33	1,224	406,900	5.6095
12	7.49	61.55	2,288	590,000	5.7709
13	7.4	56.28	2,927	689,900	5.8389
14	8.55	85.09	3,593	1,281,000	6.1075
15	7.95	68.39	4,227	1,211,000	6.0831
16	8.4	81.61	4,340	1,483,000	6.1712
17	8.71	93.10	4,696	1,832,000	6.2630
18	8.24	78.24	5,322	1,744,000	6.2417
19	—	—	—	—	—
20	8.45	81.79	7,230	2,478,000	6.3940
21	—	—	—	—	—
22	8.72	91.03	7,853	2,994,000	6.4764
23	8.55	84.47	8,580	3,036,000	6.4823
24	—	—	—	—	—
25	8.44	81.74	7,693	2,634,000	6.4202
26	—	—	—	—	—
27	8.44	85.06	7,797	2,778,000	6.4438
28	8.58	90.08	6,320	2,385,000	6.3775
29	8.59	87.18	6,753	2,466,000	6.3921
30	8.67	87.64	6,743	2,476,000	6.3937
31	9.05	101.36	8,237	3,497,000	6.5437
32	8.97	100.36	7,043	2,961,000	6.4715
33	—	—	—	—	—
34	9.04	107.55	7,437	3,359,000	6.5251
35	9.13	101.27	7,487	3,170,000	6.5019
36	9.04	98.18	7,590	3,121,000	6.4944
37	9.08	100.14	8,287	3,476,000	6.5411
38	9.51	116.59	7,724	3,772,000	6.5767
39	8.97	98.4	7,857	3,238,000	6.5104
40	—	—	—	—	—
41	9.73	125.84	8,577	4,521,000	6.6553
42	9.52	120.27	8,773	4,420,000	6.6455
43	9.96	135.18	8,653	4,900,000	6.6902
44	9.14	107.16	8,160	3,664,000	6.5640
45	9.21	107.91	10,253	4,635,000	6.6661
46	9.43	119.40	8,373	4,188,000	6.6220
47	—	—	—	—	—
48	9.15	104.83	8,453	3,712,000	6.5696
49	9.28	109.56	9,067	4,161,000	6.6192
50	9.23	110.64	9,140	4,236,000	6.6270
51	10.2	145.4	8,687	5,290,000	6.7235
52	9.46	116.94	9,120	4,467,000	6.6501

*Medium with 1 per cent. Glucose.*

<i>Day of Expt.</i>	<i>Average Diameter. μ.</i>	<i>Culture B.</i>			
		<i>R<sup>3</sup>. μ<sup>3</sup>.</i>	<i>No. of Cells per c.mm.</i>	<i>Bulk per c.mm. μ<sup>3</sup>.</i>	<i>Log. Bulk.</i>
	(7)	(8)	(9)	(10)	(11)
0	7.48	55.06	1.3	308	2.4879
1	7.95	68.71	1.3	384	2.5841
2	6.23	38.69	4.6	747	2.5845
3	7.9	62.7	9.4	2,480	3.3945
4	10.1	143.69	12.6	7,560	3.8784
5	6.75	41.21	52.5	9,060	3.9573
6	8.36	75.46	69.7	22,000	4.3432
7	8.11	79.54	123	40,900	4.6118
8	7.2	53.73	536	120,700	5.0816
9	7.97	67.24	638	192,400	5.2844
10	8.63	88.63	874	324,400	5.5112
11	7.66	67.48	1,517	428,900	5.6324
12	7.54	69.46	3,757	1,093,000	6.0387
13	7.03	48.90	4,853	994,100	5.9974
14	7.94	66.31	5,556	1,543,000	6.1884
15	7.77	62.25	6,207	1,618,000	6.2091
16	7.49	57.29	6,164	1,480,000	6.1701
17	8.2	74.93	6,990	2,194,000	6.3412
18	7.89	65.31	6,780	1,855,000	6.2683
19	—	—	—	—	—
20	8.33	79.10	8,300	2,750,000	6.4393
21	—	—	—	—	—
22	8.91	92.47	8,600	3,331,000	6.5226
23	9.21	106.47	8,883	3,962,000	6.5979
24	—	—	—	—	—
25	8.6	86.78	9,367	3,405,000	6.5321
26	—	—	—	—	—
27	8.46	81.06	9,187	3,119,000	6.4941
28	8.79	94.72	8,937	3,546,000	6.5497
29	8.84	93.71	8,323	3,267,000	6.5142
30	8.67	88.37	8,610	3,187,000	6.5034
31	8.89	95.82	8,793	3,529,000	6.5477
32	9.22	108.01	8,013	3,625,000	6.5594
33	—	—	—	—	—
34	8.78	93.49	9,257	3,625,000	6.5594
35	9.65	121.39	8,737	4,442,000	6.6477
36	9.23	105.61	9,147	4,046,000	6.6071
37	8.69	89.70	9,298	3,493,000	6.5433
38	9.34	110.70	8,551	3,965,000	6.5983
39	8.5	81.73	10,127	3,467,000	6.5400
40	—	—	—	—	—
41	8.61	87.19	11,347	4,143,000	6.6175
42	8.67	90.73	12,200	4,637,000	6.6662
43	9.35	111.34	11,850	5,528,000	6.7426
44	8.39	82.09	12,347	4,246,000	6.6280
45	7.93	70.91	11,987	3,479,000	6.5415
46	8.5	89.61	13,790	5,590,000	6.7479
47	—	—	—	—	—
48	8.43	87.35	17,370	6,355,000	6.8031
49	8.18	77.91	16,300	5,320,000	6.7260
50	8.36	84.54	16,247	5,752,000	6.7599
51	8.45	86.1	19,420	7,009,000	6.8457
52	—	—	—	—	—

TABLE III.

*Number of Autospores produced by Dividing Cells.*

<i>Culture.</i>	<i>Day of Expt.</i>	<i>No. of Cells with 4 Autospores. %</i>	<i>No. of Cells with 8 or 16 Autospores. %</i>	<i>No. of Cells recorded.</i>	
Observations made during period of constant growth rate	A	9	77	23	75
		10	79	21	66
	B	9	75	25	112
		10*	84	16	51
	X	11	85	15	74
	Y	9†	83	17	95
Total for the six observations			80	20	473
Observations made during period of falling growth rate	A	32	52	48	237
		37	41	59	150
	B	11*	42	58	85
		32	—	52	174
		37	43	57	94
	X	13	56	44	259
	Y	10†	64	36	205
		12	60	40	149
		14	54	46	267
	Total for the nine observations			53	47

\* Consecutive days, same culture. †Ditto.

An interesting application of this observation may be made in the case of the experimental value obtained for culture A in this experiment after eight days' growth. It is seen from Text-fig. 3 that this point is considerably below the straight line of nearest fit, but it can be shown that this low position is largely due to a sampling error.

On day 7 there were 700 cells in 10 drops.

„ 8 „ 2,315 „ 10 „

∴ increment = 1,615 cells.

Observations indicate that during the logarithmic period, of dividing cells { 80 per cent. divide into 4,  
20 „ „ 8.

Let  $x$  represent number of cells dividing into 8 and becoming  $8x$  cells. Then  $4x$  will represent number of cells dividing into 4 and becoming  $16x$  cells.

$$\therefore 2315 = 24x + (700 - 5x)$$

$$\text{whence } x = 85,$$

i.e. { 85 cells divided into 8, becoming 680 } total 2040,  
340 „ „ „ 4, „ 1360

and 275 remained undivided and would be large.

∴ one might expect  $\frac{275 \times 50}{2315}$  large cells out of 50 cells drawn  
 = 6 large cells.

But of 50 cells drawn, only 4 were large.

Substitute two large cells, diam.  $11.5\mu$  and  $12\mu$  respectively,

$$\text{i. e. } r^3 = 185.19 + 216\mu^3 = 401.19\mu^3$$

for two small cells, diam.  $5\mu$  and  $5.5\mu$  respectively,

$$\text{i. e. } r^3 = 15.63 + 19.68\mu^3 = 35.31\mu^3$$

$$\text{Difference} = 365.88\mu^3$$

$$\therefore \text{total } r^3 \text{ for 50 cells} = 2064.73 + 365.88\mu^3$$

$$= 2030.61\mu^3$$

$$\therefore \text{average } r^3 = 48.61\mu^3$$

$$\text{and bulk of alga in 10 drops} = \frac{4}{3}\pi \times 48.61 \times 2315\mu^3$$

$$\therefore \text{Bulk per c.mm.} = 52.380\mu^3$$

$$\text{Log. bulk} = 4.7192.$$

This adjustment of the calculated value would about halve the error of the observed point, and would bring it reasonably near to the straight line of nearest fit, as is indicated by the small + sign in Text-fig. 3.

It appears then that the conditions which arise in the culture, and cause the falling off of the growth rate after a certain period of time, are such that they delay the multiplication of the cells and increase the number of autospores produced. Death of individual cells in the cultures was not observed during the period of constant growth rate. The decrease in the growth rate was observed to be largely due, however, to the death of an increasing proportion of the autospores after they had been set free from the mother-cell; they lost colour and completely disintegrated.

The fluctuations in bulk of the alga towards the end of the experiment were the outcome of the ratio of survivors to the number of autospores produced, and the gradual upward trend of the logarithmic curve was due in part to the gradual increase in average size of the cells, and in part to the fact that, on the whole, the number of autospores that survived was slightly greater than the number of mother-cells that divided.

A comparison of the two curves plotted in Text-fig. 3 brings out the fact that the two parallel cultures behaved in a very similar manner throughout the course of the experiment, though the values for culture B were slightly higher than those for culture A most of the time. For the early part of the logarithmic curve it is easy to calculate algebraically the straight line which most nearly fits the observed points, and to determine the degree of variance of these points from the calculated line.<sup>1</sup> Omitting the first point from both series of values, since it is obviously exceptional, it is found that the

<sup>1</sup> An example of the method by which these calculations are made is given in detail in section C (i), dealing with the growth of the organism in a medium containing galactose.



straight line of nearest fit to the next ten values of culture A is represented by the equation  $y = 0.31x + 3.276$ , and that to the next nine values of culture B by  $y = 0.339x + 3.26$ . The difference in slope of the two lines is then  $0.339 - 0.31$ , a difference of 29 in 339, or approximately 8.5 per cent., in the rate of growth. The standard error was calculated for the two cultures from the variance of the observed points from the calculated straight lines, and was found to be only slightly less than the difference in slope between the two curves. It was therefore concluded that the difference in rate of growth of the two cultures was not significant, and that *equal amounts of a uniform suspension of the organism inoculated into parallel cultures grow at the same rate.*

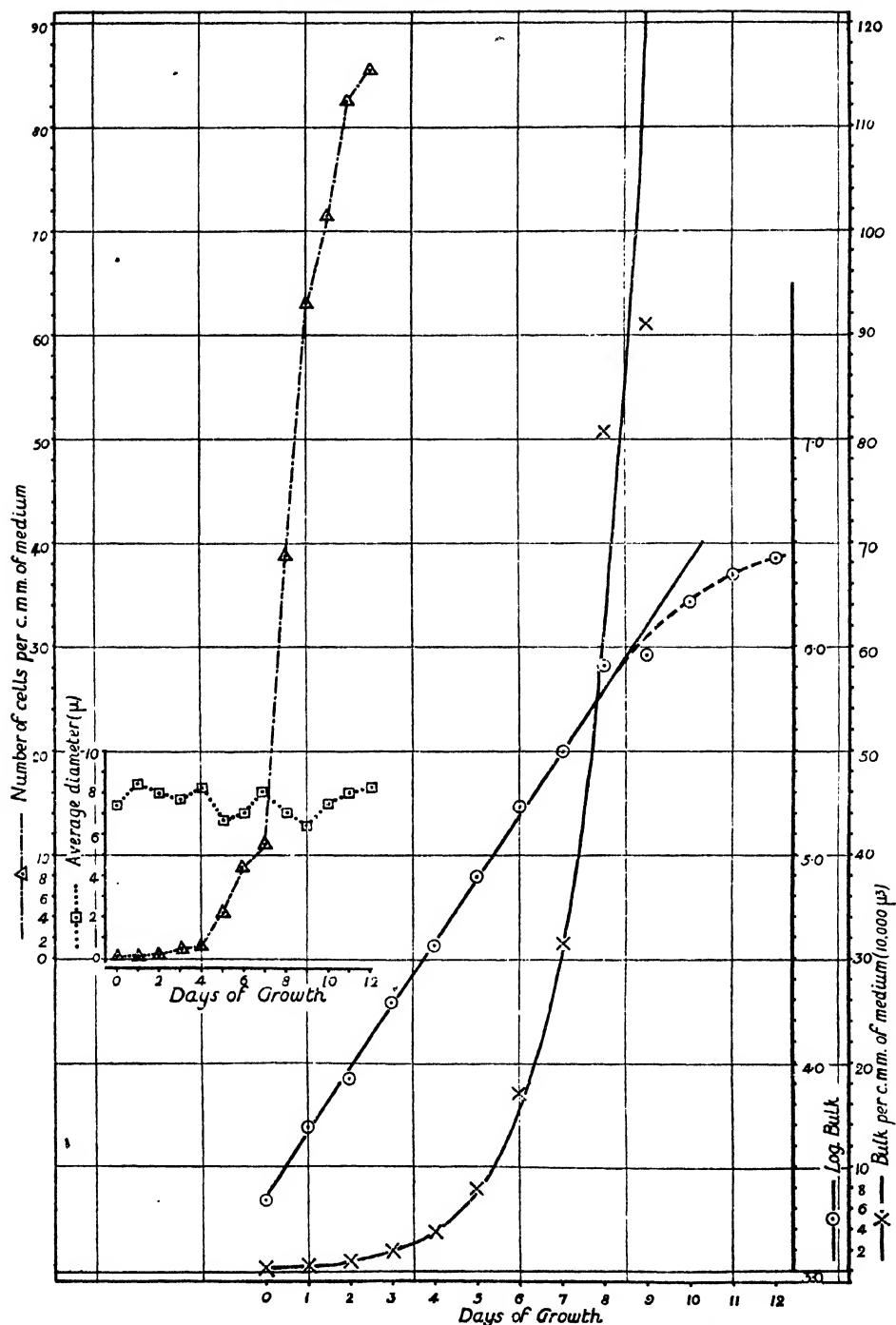
If, then, equal amounts of a uniform suspension of cells are inoculated into cultures differing in respect of one factor, it should be possible, by determining the rate of growth in the two sets of conditions, to measure the effect of the varying factor; a number of different factors have therefore been studied from this point of view. Owing to the fact that after nine or ten days, in the conditions under which these cultures were grown, the rate of growth has always been observed to fall off rapidly, observations have not usually been made for a longer period than ten days, since that was thought to be sufficient to determine, with a fair degree of accuracy, the initial slope of the logarithmic curve.

Since this method of investigation depends on the assumption that the growth rate of the organism in the glucose medium in the light, under constant conditions, is uniform for a limited period of time, and since the rate of growth in a given medium is known to vary with the condition of the mother culture, a control culture in the glucose medium has been observed in every experiment, to provide a standard for comparison and to accumulate evidence of the truth of the original assumption. Duplicate cultures embodying the factor under consideration have always been set up.

#### (ii) *Growth in a Well-aerated Culture.*

It has been stated above that the more carefully are the external conditions affecting the cultures kept constant, the more nearly do the logarithmic values of the bulk lie on a straight line, provided that the conditions are favourable to growth.

This fact has been strikingly demonstrated by a number of cultures carried out in large distillation flasks (2–5 litres) in which the liquid was kept in constant circulation by drawing air continuously through the medium by means of a filter-pump. The projecting stem of the calcium chloride tube filled with cotton-wool was fitted with a glass tube finely drawn out and projecting to within a few millimetres of the bottom of the liquid;



TEXT-FIG. 4. Diagram showing uniform rate of growth of *Scenedesmus* sp. in a constantly aerated liquid medium containing mineral salts + 1 per cent. glucose: for explanation see text.

a short bent glass tube through a second hole in the rubber stopper of the flask provided the means of attachment to the filter-pump. Both inlet and outlet tubes were well plugged with sterile cotton-wool to prevent contamination of the culture.

The results for the first twelve days of one of these cultures are given in Table IV and plotted in Text-fig. 4.

TABLE IV.

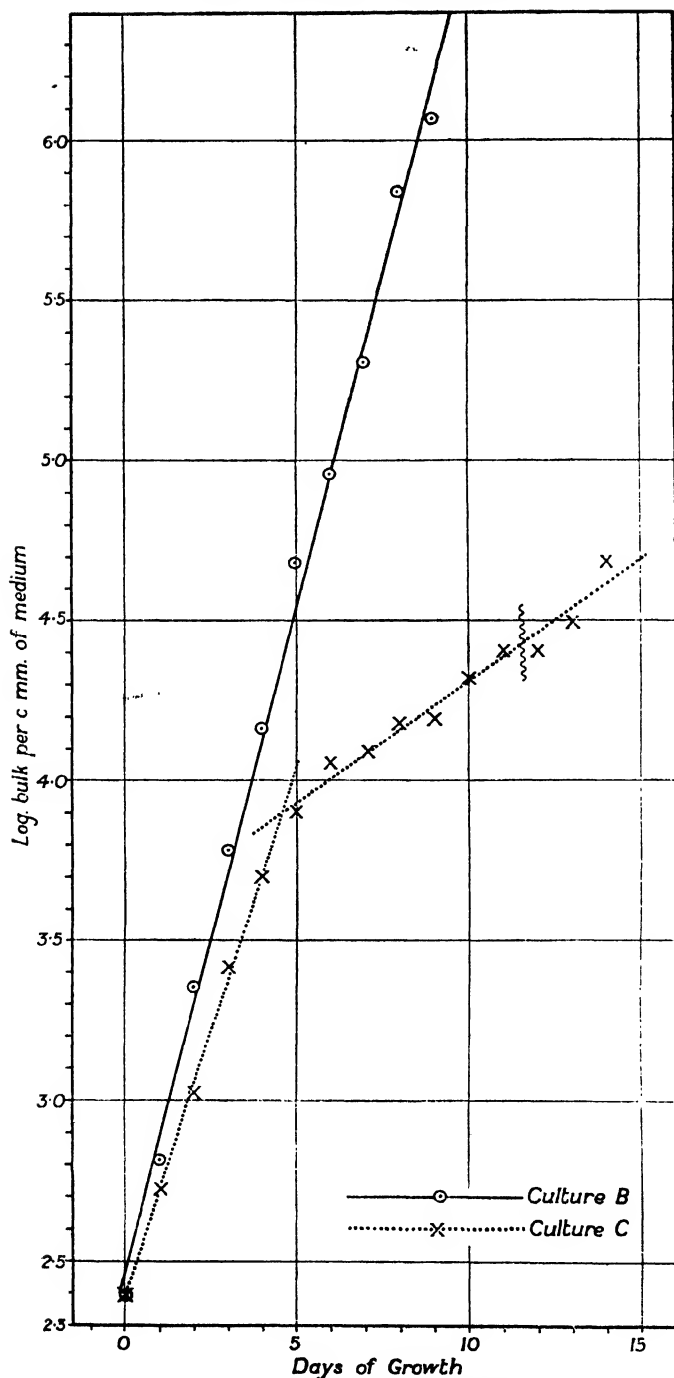
*Growth in a Well-aerated Culture.*

<i>Day of Expt.</i>	<i>Average diam. μ.</i>	<i>Average r<sup>3</sup>. μ<sup>3</sup>.</i>	<i>Per c.mm. No. of Cells.</i>	<i>Bulk. μ<sup>3</sup>.</i>	<i>Log. Bulk.</i>
0	7.38	51.45	10	2,155	3.3334
1	8.4	76.84	15	4,829	3.6838
2	7.97	72.05	27	8,270	3.9175
3	7.67	62.2	73	19,100	4.2810
4	8.2	76.77	112	36,020	4.5565
5	6.58	42.71	437	78,170	4.8931
6	6.97	46.65	867	169,400	5.2290
7	7.93	68.75	1,088	313,400	5.4961
8	7.03	49.7	3,880	807,700	5.9073
9	6.32	34.59	6,283	910,300	5.9592
10	7.44	54.89	7,150	1,644,000	6.2159
11	7.92	64.78	8,248	2,238,000	6.3498
12	8.22	73.9	8,558	2,649,000	6.4231

The diagram shows very clearly the irregularity of the increase in number of cells per unit volume of medium, and the very considerable fluctuations in average diameter of the cells from day to day. The first eight logarithmic values of the bulk calculated from these data, on the contrary, lie almost exactly on the calculated straight line of nearest fit to the first ten values, viz.  $y = 0.305x + 3.353$ , showing that the relative rate of increase in bulk was approximately constant during this period, while after nine days the rate of growth gradually decreased in the manner indicated by the broken line.

The almost mathematical regularity with which the organisms increased in bulk in this experiment is further demonstrated in the diagram by plotting the calculated actual values of the bulk and drawing through them the theoretical exponential curve corresponding to the straight line of nearest fit to the logarithms. The extreme closeness of the first eight points to this mathematical line is very striking, and it is from experimental data of this kind that the writer has become convinced that the growth rate of a culture of this organism is constant under uniform favourable conditions.

Constant aeration of all the cultures under observation has not been practicable, since the increased accuracy of the data so obtained has been



TEXT-FIG. 5. Diagram showing rate of growth of *Scenedesmus* sp. in an anaerated medium containing mineral salts alone (culture C) compared with that in the same medium to which glucose has been added (culture B) : for explanation see text.

found to be more than counterbalanced by the technical difficulties of sampling, and the increased risk of contamination of the cultures.

(iii) *A Comparison of Cultures grown with and without Glucose.*

(a) Three unaerated cultures were set up, two (A and C) with a solution of mineral salts, and the third (B) with the same solution containing in addition 1 per cent. glucose. They were inoculated in the order A, B, C, with 0.71 c.c. each of a counted suspension of cells; they were thoroughly shaken and placed in the water bath at 24.4° C. in the dark room with a constant illumination. Unfortunately, flask A met with an accident on the third day of the experiment, and the culture had to be discarded. This is particularly unfortunate, as the results may be interpreted in more than one way. A single culture only is therefore available for comparison with the glucose medium; the agreement between parallel cultures in other media to be described later is so close, however, that it has been decided to include the experiment in the series.

TABLE V.

*Culture B, containing 1 per cent. Glucose (unaerated).*

<i>Day of Expt.</i>	<i>Average Diam. μ.</i>	<i>R<sup>s</sup>. μ<sup>s</sup>.</i>	<i>Number per c.mm.</i>	<i>Bulk per c.mm. μ<sup>s</sup>.</i>	<i>Log. Bulk.</i>
0	6.9	45.6	1.3	248	2.3950
1	8.8	88.2	1.8	665	2.8229
2	8.4	84.4	6.4	2,260	3.3548
3	10.0	133.4	10.8	6,035	3.7807
4	8.0	75.1	46	14,470	4.1605
5	9.3	113.5	100	47,540	4.6771
6	7.4	56.02	384	90,110	4.9548
7	7.7	63.3	760	201,500	5.3043
8	7.2	52.9	3107	688,600	5.8380
9	7.5	58.1	4800	1,168,000	6.0676

*Culture C, Mineral Salts alone (unaerated).*

0	6.9	45.6	1.3	248	2.3950
1	8.1	74.4	1.7	530	2.7241
2	6.8	44.0	5.7	1,050	3.0215
3	7.9	66.2	9.4	2,610	3.4161
4	7.4	56.5	21	4,970	3.6963
5	7.4	52.7	36	7,950	3.9002
6	7.5	55.0	49	11,300	4.0527
7	7.8	60.0	49	12,330	4.0908
8	8.3	73.7	49	15,130	4.1798
9	8.5	77.7	48	15,620	4.1937
10	8.6	83.4	59	20,610	4.3142
11	8.9	90.5	68	25,800	4.4112

Filament of lamp burned out during night.

12	8.4	79.4	77	25,610	4.4084
13	8.1	71.3	104	31,060	4.4922
14	8.2	73.5	156	48,030	4.6815

Observations on the glucose medium were discontinued after nine days' growth, but the culture in mineral salts alone appeared to be growing so slowly that records were made until the fourteenth day. There was a discontinuity in the experiment, however, because the filament of the electric lamp burned out at some time on the eleventh day, so that the flask was in darkness for an unknown period of time. The records of the two cultures are given in Table V, and the logarithmic values of the daily bulk plotted against time in Text-fig. 5.

These two cultures provide convincing evidence of the absence of a lag period at the beginning of the experiment, and of the uniformity of the rate of growth during the first few days. The first nine logarithmic values of the bulk of the alga in the glucose medium, and the first five values for the mineral salts medium, lie approximately upon the calculated straight lines:

$$y = 0.412x + 2.481 \text{ for the glucose culture,}$$

$$\text{and } y = 0.3295x + 2.39 \text{ for the mineral salts medium.}$$

After the fifth day the observed values of the bulk in the mineral salts solution lie scattered about the calculated straight line

$$y = 0.0758x + 3.55.$$

More information is required on this subject, but of a number of possible explanations of the abrupt change in direction of the line, two are the most likely. It might be that the cells of the mother culture contained a certain amount of reserve food in the form of starch, and that this was used up during the first few days of rapid growth after inoculation into the liquid medium, which appears to provide more favourable conditions for growth than an agar medium. After the reserve starch had been used up, the cells would be completely dependent on the supply of carbon dioxide for their nutrition, and the rate of increase in bulk would be lessened. Or it might be that there was a deficiency of available  $\text{CO}_2$  in the later stages of the experiment. As stated above, the medium absorbs  $\text{CO}_2$  from the air very readily if left loosely plugged in the laboratory, but this initial supply might be used up fairly rapidly, and further absorption be considerably impeded by the length of the cotton-wool plug ( $5\frac{1}{2}$  inches), through which the  $\text{CO}_2$  would have to diffuse.

If the first explanation be the correct one, then the rate of growth in the mineral salts medium was really represented by the slope of the second part of the line, viz. 0.0758, as compared with the slope of the glucose line, viz. 0.412. In other words, the rate of growth in the mineral salts solution was only  $76/412$ , or about 18 per cent. of the rate when 1 per cent. glucose was added, a figure which agrees quite reasonably with the observations on solid media.

If, on the other hand, the second explanation be more correct, then

the slope of the first part of the line, viz. 0.3295, must be regarded as the true measurement of the rate of growth in the mineral salts medium, which would then be  $329.5/412$  or about 80 per cent. of that in the glucose medium. This is not in accordance with other observations on solid media, but it is possible that in these cultures, too, the rate of growth may be limited by the rate at which  $\text{CO}_2$  diffuses through the cotton-wool plugs.

(b) A second experiment was carried out in 1-litre flasks in which the media were kept continuously aerated to ensure a continuous supply of  $\text{CO}_2$  to the growing organisms. The cultures were set up each with 400 c.c. of medium, two with mineral salts alone, and the third with 1 per cent. glucose in addition. They were placed in the same water bath at  $24.6^\circ\text{C}$ . and kept under constant illumination. A fourth culture, with mineral salts alone, was left unaerated and placed in daylight in a second water bath at the same temperature; this became contaminated at an early date, and therefore had to be discarded.

The logarithmic values of the bulk for the three aerated cultures are given in Table VI and plotted in Text-fig. 6.

TABLE VI.

*Bulk of Alga per c.mm. of Medium (log. values) in Aerated Cultures containing Mineral Salts alone and the same + 1 per cent. Glucose.*

Day of Expt.	Mineral Salts alone.		Glucose
	Culture B.	Culture C.	Culture D.
0	2.3010	2.3010	2.3617
1	2.8021	2.7627	2.8344
2	3.0191	2.8927	3.1872
3	3.3369	3.2546	3.7270
4	3.4914	3.5024	4.1886
5	3.7837	3.7238	4.5911
6	4.2014	4.1139	5.0653
7	4.4106	4.2258	5.3918
8	4.4683	4.4264	5.7707
9	4.7965	4.7812	6.3205

It is seen from the diagram that the ten values for the glucose culture all lie very close to the calculated straight line of nearest fit to them, viz.

$$y = 0.434x + 2.39.$$

The values for the mineral salts cultures, though obtained with equal care, show a considerably greater degree of deviation from a straight line. It is extremely interesting to find, however, that the two lines of closest fit to the experimental values, calculated separately for each culture, have practically the same slope, viz. 0.266 as against 0.263; that is to say, the average growth in the two parallel cultures was the same over the

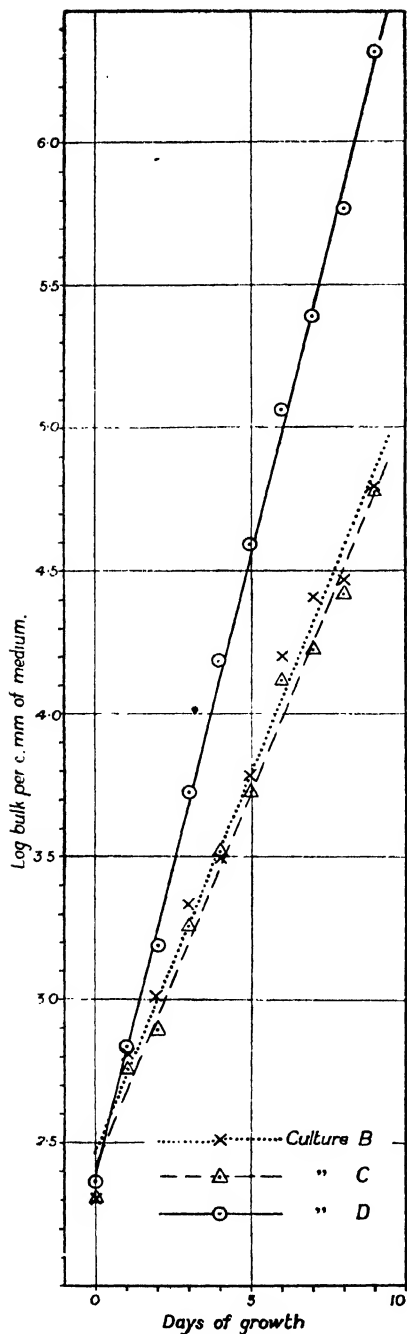


FIG. 6.

TEXT-FIG. 6. Diagram showing rate of growth of *Scenedesmus* sp. in a medium containing mineral salts alone (cultures B and C) compared with that in the same medium to which glucose has been added (culture D), all the cultures being continuously aerated: for explanation see text.

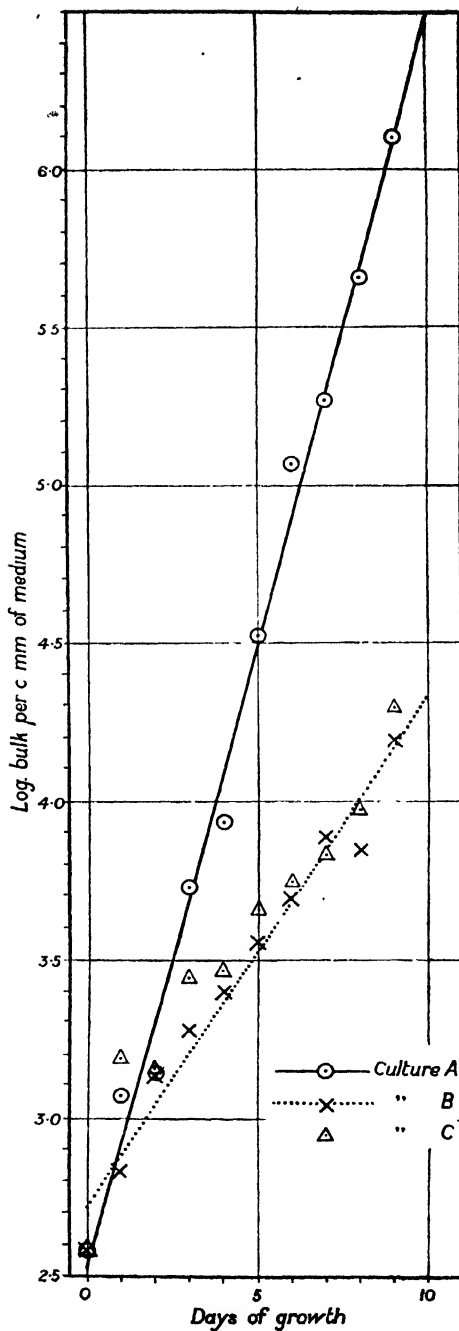


FIG. 7.

TEXT-FIG. 7. Diagram to show relative rates of growth of *Scenedesmus* sp. in a liquid medium containing glucose in the light (culture A) and in complete darkness (cultures B and C): for explanation see text.



period observed. These two lines are indicated in the diagram (broken lines), their equations being

$$y = 0.266 x + 2.464 \dots\dots \text{for culture B,}$$

$$y = 0.263 x + 2.415 \dots\dots \text{for culture C.}$$

There is no suggestion from the values obtained for these cultures of the sudden retarding effect of a limiting factor in the mineral salts medium, such as was observed in the unaerated cultures, the average growth rate throughout the period of observation being  $0.265/0.434$ , or 61 per cent., of that in the aerated glucose medium. This is considerably lower than the figure, 80 per cent., obtained for the first part of the line in the unaerated cultures, but the conditions of the two experiments were not identical, for the greatly increased supply of oxygen due to continuous aeration is likely to have a considerable effect on the growth of the organism both in the presence and in the absence of glucose.

It appears from the two experiments, therefore, that the rate of growth of the organism in a mineral salts medium is limited by the supply of  $\text{CO}_2$ , and that under favourable conditions of growth it is about 60 per cent. of that in the presence of glucose.

(iv) *The Growth of Scenedesmus costulatus, var. chlorelloides, in the dark.*

Three parallel cultures were set up with a medium of mineral salts + 1 per cent. glucose and inoculated each with 0.1 c.c. of a suspension of algal cells taken from a two months old culture on glucose agar. Culture A was placed in a water bath at  $24.4^\circ \text{C.}$  under controlled light; cultures B and C were placed in a similar water bath at  $24.4^\circ \text{C.}$ , in a special dark room. The only light allowed was that from a red electric bulb which was switched on for a few minutes during the daily manipulation of the cultures.

Seeing that in the case of the cultures grown in the dark there was a temperature fluctuation of  $1^\circ \text{C.}$ , as compared with  $0.1^\circ \text{C.}$  in the rest of the experiments, the results obtained with these cultures have not the same quantitative significance as the other results of the series. The experiment, however, establishes the important fundamental fact that the alga *Scenedesmus costulatus, var. chlorelloides*, can grow in complete darkness.

The observed and calculated values are given in Table VII, and the logarithmic values of the bulk are plotted against time in Text-fig. 7, where the dotted line represents the calculated straight line of nearest fit to the values for culture B.

It is seen that the two cultures in the dark grew at about the same rate and in the same way; in both, the average size of the cells steadily increased, and there was a slow but steady increase in number of the cells.

The rate of growth was considerably below that in the control culture in the light, and was by no means uniform during the course of the experiment.

TABLE VII.

*Growth in the Light and in Complete Darkness.*

*Culture A, in Light.*

Day of Expt.	Average Diam. $\mu$ .	$R^3$ , $\mu^3$ .	No. of Cells per c.mm.	Bulk per c.mm. $\mu^3$ .	Log. Bulk.
0	7.2	51.3	1.8	387	2.5875
1	8.3	76	3.7	1,178	3.0712
2	7.8	65.3	5.1	1,395	3.1446
3	8.6	85.8	15	5,391	3.7317
4	7.4	61.1	34	8,702	3.9396
5	8.5	86	93	33,500	4.5250
6	8.8	104.2	267	116,500	5.0664
7	7.7	63.4	693	184,000	5.2648
8	8	75.7	1,420	450,300	5.6535
9	7.9	67.1	4,446	1,250,000	6.0968

*Cultures in Dark.*

*Culture B.*

0	7.2	51.3	1.8	387	2.5875
1	7.9	67.8	2.4	682	2.8335
2	8.7	95.1	3.4	1,354	3.1316
3	8.4	84.6	5.4	1,914	3.2819
4	8.7	89	6.8	2,535	3.4040
5	9.2	104.6	8.3	3,637	3.5607
6	9.3	112.4	10.4	4,897	3.6899
7	10	132	14	7,741	3.8888
8	10	136.2	12.5	7,131	3.8532
9	10	139.4	27	15,770	4.1978

*Culture C.*

0	7.2	51.3	1.8	387	2.5875
1	8	68.1	5.4	1,540	3.1879
2	8.3	78.9	4.3	1,421	3.1526
3	8.6	89.4	7.4	2,771	3.4427
4	9.1	102.5	6.8	2,905	3.4631
5	9	101	10.8	4,569	3.6599
6	9.4	116.31	11.5	5,602	3.7484
7	9.3	107.5	15	6,756	3.8297
8	10.2	150.1	15	9,431	3.9745
9	10.7	168.3	28	19,740	4.2954

The average rate of growth of culture B in the dark is represented by the straight line whose equation is

$$y = 0.162x + 2.714,$$

whereas that for culture A in the light is

$$y = 0.393x + 2.54;$$

whence it follows that the rate of growth in the dark was roughly forty per cent. of that in the light.

Although, as stated above, these cultures have not the same quantita-

tive significance as the other experiments, this figure, 40 per cent., is very suggestive, for the rate of growth in the aerated mineral salts medium in the light was found to be about 60 per cent. of that in the glucose medium under the same conditions. The two experiments taken together seem to indicate that the organism in the glucose medium in the light carries on normal photo-synthesis and the assimilation of glucose independently of one another. Further evidence is required, however, before this fact can be regarded as established.

It follows directly from this experiment that the absence of light need not prevent the growth of the organism in the soil, provided that a suitable supply of organic food is available for its use. It is interesting to note that these cultures were deep green in colour at the end of seven weeks in complete darkness.

#### C. GROWTH IN MEDIA CONTAINING CERTAIN OTHER SOLUBLE ORGANIC COMPOUNDS COMPARED WITH THAT IN GLUCOSE.

The following compounds were selected for study :

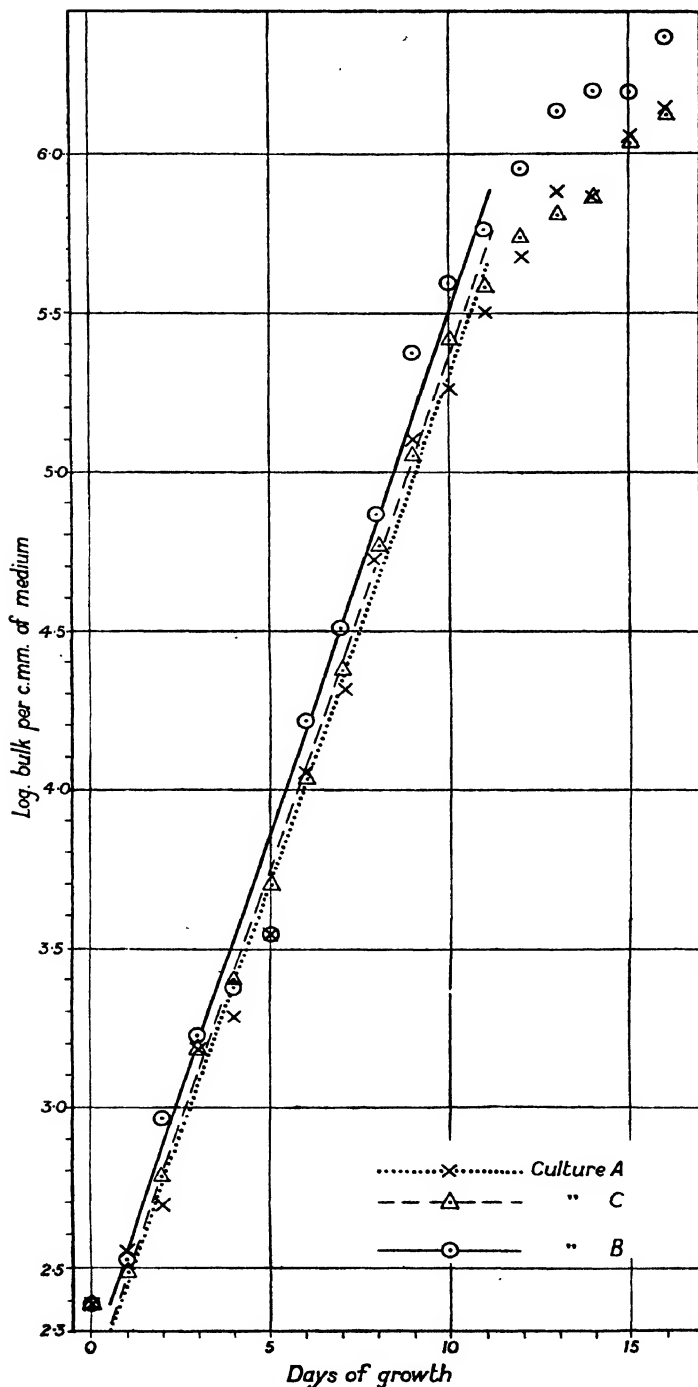
hexoses—galactose and fructose.  
disaccharoses—sucrose and maltose.  
pentose—xylose.  
triose—glycerine.  
alcoholic derivative of hexose—mannite.

Three cultures made from the same solution of mineral salts, one containing 1 per cent. glucose and two containing 1 per cent. of the compound to be investigated, were inoculated with an approximately equal number of cells (1.3 cells per c.mm.) and placed in the same water bath in dim daylight or under controlled electric light. Since other conditions were uniform for the three cultures, any difference in growth rate must be due to the nature of the organic compound present.

##### (i) *Growth in a Medium containing Galactose.*

This was one of the first experiments carried out, and the experimental error of the earlier observations was high. It affords an interesting series of data, however, for the demonstration of the statistical methods<sup>1</sup> that have been used in examining the results of these experiments, and it is therefore dealt with in considerable detail.

<sup>1</sup> The writer is indebted to Mr. R. A. Fisher, head of the Statistical Department, for his assistance in devising a method for testing the significance of these results. The theory underlying the method is discussed in chapter v of his book, *Statistical Methods for Research Workers* (10).



TEXT-FIG. 8. Diagram to show relative rates of growth of *Scenedesmus* sp. in liquid media containing galactose (cultures A and C) and glucose (culture B); for explanation see text.

The calculated logarithmic values of the bulk of alga per cubic millimetre of medium in each of the three cultures are given for seventeen days in Table VIII and plotted in Text-fig. 8.

TABLE VIII.

*Bulk of Alga per c.mm. of Medium (logarithmic values) in Cultures containing Galactose and Glucose.*

<i>Day of Expt.</i>	<i>Culture A.</i>	<i>Galactose.</i> <i>Culture C.</i>	<i>Glucose.</i> <i>Culture B.</i>
0	2.3822	2.3822	2.3822
1	2.5495	2.4880	2.5222
2	2.6960	2.7862(?)	2.9631
3	3.1780	3.1859	3.2269
4	3.2869	3.4023	3.3744
5	3.5467	3.7053	3.5401
6	4.0527	4.0401	4.2174
7	4.3221	4.3789	4.5074
8	4.7117	4.7689	4.8695
9	5.1050	5.0558	5.3712
10	5.2631	5.4135	5.5953
11	5.5089	5.5808	5.7037
12	5.6773	5.7392	5.9538
13	5.8781	5.9106	6.1349
14	5.8650	5.8619	6.1999
15	6.0566	6.0349	6.1931
16	6.1405	6.1329	6.3682

It is seen from the figure that the first point, i. e. the supposed bulk of the inoculum, does not lie near any straight line that can be drawn through the rest of the points, and it is thought that either too little of the suspension was added or that the mother culture contained a number of moribund or resting cells. This value has therefore been omitted from the calculations for all three cultures.

The following equations represent the straight lines of nearest fit to the observed values calculated algebraically for the three cultures taken separately.

$$\begin{array}{ll}
 \text{Galactose cultures} & \left\{ \begin{array}{l} A, y = 0.317x + 2.13 \\ C, y = 0.322x + 2.15 \end{array} \right. \\
 \text{Glucose culture} & B, y = 0.344x + 2.127
 \end{array}$$

When the lines represented by these equations are drawn through the observed points, it is seen that the observed values for cultures A and C lie as closely as could be expected to the calculated straight lines, while those of culture B are more widely divergent than is usual in cultures containing

glucose. The standard error of the observations can be calculated by summing the squares of the deviations of the observed points from the calculated line, the amount of the deviation of each point ( $y - Y$ ) being read off from the diagram. The cultures are then compared in the following way :

For culture A	$S(y - Y)^2 = 0.0781$
For culture C	$S(y - Y)^2 = 0.0152$
For the two cultures taken together	$S(y - Y)^2 = 0.0933$

But  $q$  being the number of degrees of freedom from the mean (i.e. 8 for each curve)

$$S(y - Y)^2 = q S^2$$

$$\text{i. e. } q S^2 = 0.0933$$

$$S^2 = \frac{0.0933}{16}$$

$$= 0.005831$$

And variation of regression coefficient (where  $n$  is the number of observations)

$$S^2 / \frac{n(n^2 - 1)}{12}$$

$$= S^2 / 82.5 \text{ for each culture.}$$

i. e. Variation of regression

coefficient for A

$$= 0.0000707$$

" " C

$$= 0.0000707$$

$\therefore$  Variance of their difference

$$= 0.0001414$$

$\therefore$  Standard error

$$= \sqrt{0.0001414}$$

Observed divergence between the lines

$$\begin{cases} = 0.0119 \\ = 0.322 - 0.317 \\ = 0.005 \end{cases}$$

i. e. the observed divergence between the lines for the two galactose cultures is less than one-half of the standard error of the observations, and the rates of growth of the cultures are therefore not significantly different.

Again, for cultures A and C together

$$S(y - Y)^2 = 0.0933$$

For culture B

$$S(y - Y)^2 = 0.1573$$

For the three cultures together

$$q S^2 = 0.2506$$

$$S^2 = \frac{0.2506}{24}$$

$$= 0.010442$$

$$\text{Variation of regression coefficient for B} = \frac{S^2}{82.5} = 0.0001241$$

$$\text{" " " " A and C} = \frac{S^2}{165} = 0.0000633$$

∴ Variance of their difference	= 0.0001874
∴ Standard error	= 0.0137
Observed difference between B and the average of A and C	= 0.344 - 0.320 = 0.024

i.e. the observed difference in rate of growth between the glucose culture and the mean of the galactose cultures is a little less than twice the standard error. The probability that this result might be obtained by chance is about 1 in 10, and though the difference is very suggestive it cannot be regarded as definitely significant. It has been found by other methods, however, that cultures of the alga with glucose invariably grow a little better than those with galactose (see p. 156), and it is probable that this visible difference in final bulk is the outcome of the accumulative effect of a very small difference in rate of growth. The rate of growth in the galactose medium, as indicated by this experiment, is 0.32/0.344, or 93.5 per cent. of that in the glucose medium.

(ii) *Growth in a Medium containing Fructose.*

In view of the great similarity in structure of the molecules of the three sugars, glucose, galactose, and fructose, and of their generally similar biological reactions, it is interesting to find that fructose provides a much less favourable medium for the growth of the alga under investigation than

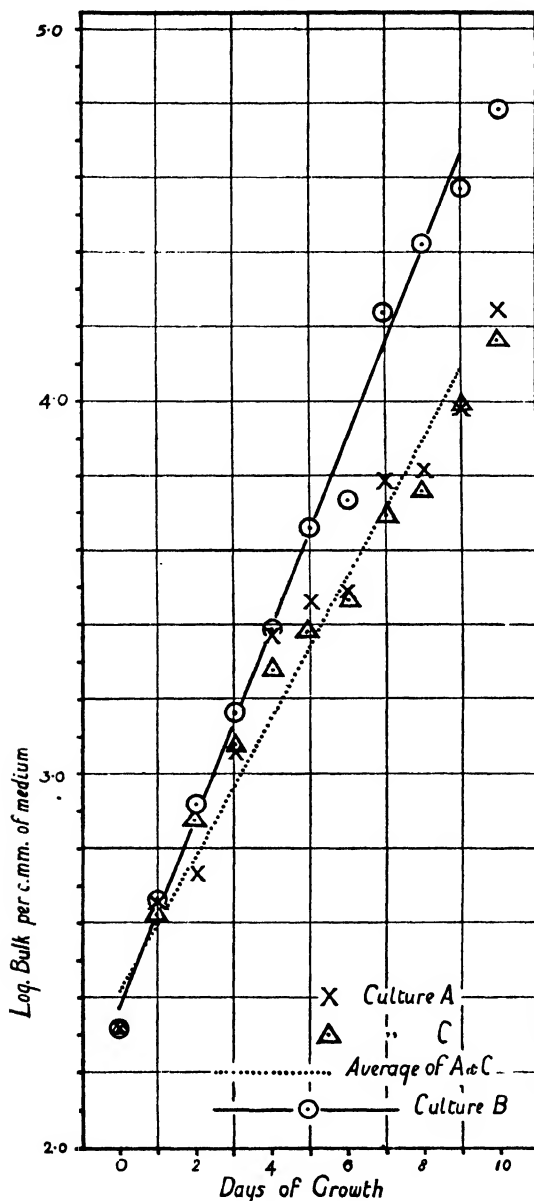
TABLE IX.

*Bulk of Alga per c.mm. of Medium (log. values) in Cultures containing Fructose and Glucose.*

Day of Expt.	Fructose.			Glucose. Culture B.
	Culture A.	Culture C.	Log. Average Bulk in A and C.	
0	2.3149	2.3149	2.3149	2.3149
1	2.6540	2.6247	2.6395	2.6623
2	2.7360	2.6886	2.7126	2.9189
3	3.06*	3.08*	3.03*	3.16*
4	3.3785	3.2813	3.3326	3.3844
5	3.4627	3.3587	3.4138	3.6606
6	3.4880	3.4621	3.4751	3.7364
7	3.7895	3.7011	3.7475	4.2393
8	3.8160	3.7600	3.7889	4.4264
9	3.9886	3.9986	3.9935	4.5692
10	4.2473	4.1654	4.2082	4.7820

\* Calculated mean of 2 and 4.

do glucose and galactose, and that cultures grown on solid media containing this sugar show a marked tendency to undergo 'staling'.

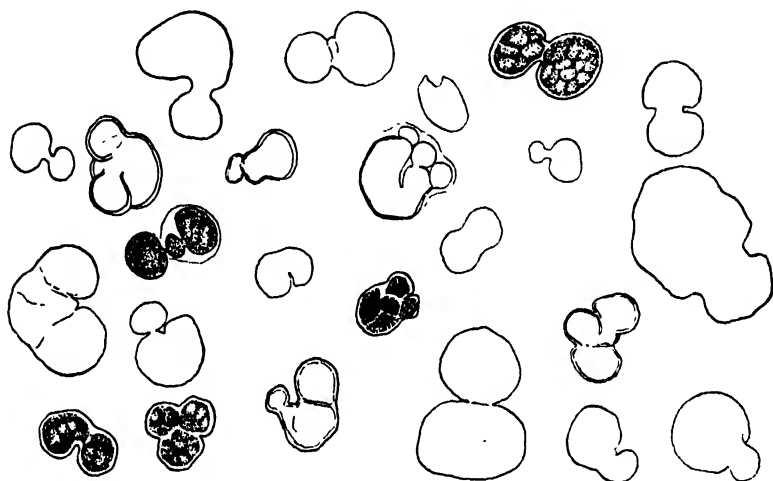


TEXT-FIG. 9. Diagram to show relative rates of growth of *Scenedesmus*, sp., in liquid media containing fructose (cultures A and C) and glucose (culture B): for explanation see text.

The logarithmic values of the algal bulk in the cultures to be compared are given in Table IX and plotted in Text-fig. 9.



The values for the glucose medium lie fairly well on the calculated straight line of nearest fit to the first nine points, viz. the line  $y = 0.255x + 2.37$ ; after this period the growth rate begins to decrease. The values for the two fructose cultures, on the other hand, are badly scattered, and it appears doubtful from the figure whether a straight line can really be considered to represent them. For the first five values, both of the cultures followed very closely the slope of the glucose curve, but later values diverged considerably from those in the glucose medium, while agreeing fairly well with each other. The curves, on the whole, are reminiscent of those for the glucose

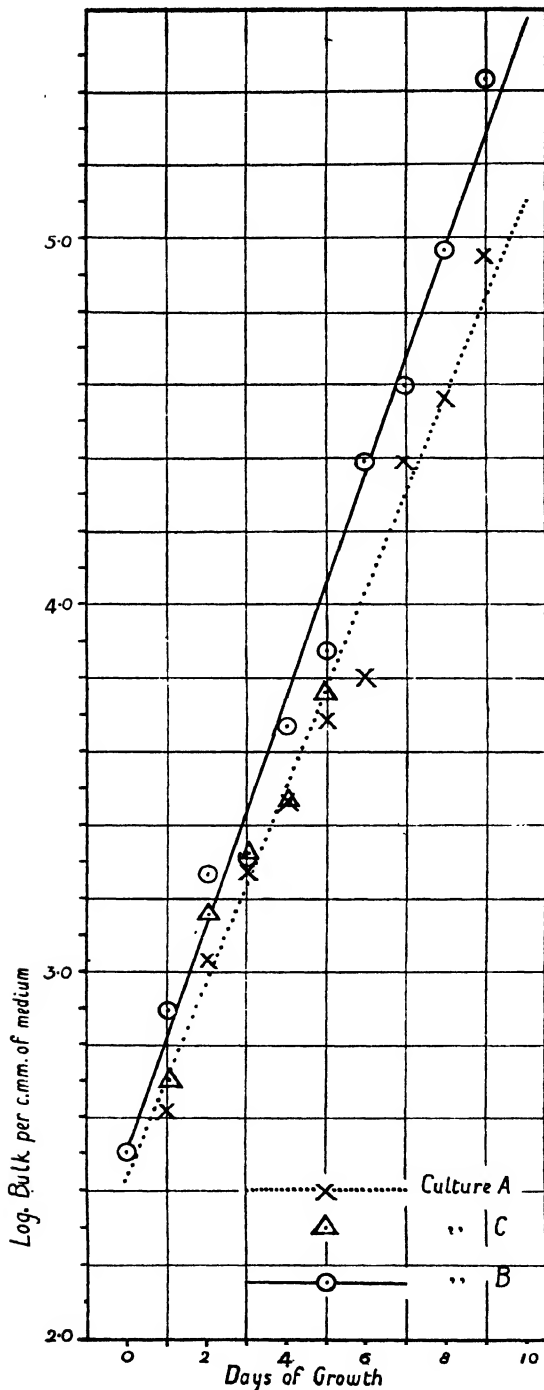


TEXT-FIG. 10. Abnormal modes of multiplication of *Scenedesmus costulatus*, Chod., var. *chlorelloides*, nov. var., in a liquid medium containing fructose.  $\times 540$  approx.

cultures grown in the dark (Text-fig. 7), and a further parallelism is noted in the fact that the average diameter in the two fructose cultures increases steadily from  $6.6 \mu$  to  $11 \mu$  and  $10.8 \mu$  respectively, instead of fluctuating in the normal manner. The numbers increased in the fructose cultures from  $1.3 \mu$  to  $23 \mu$  and  $19 \mu$  respectively per c.mm., figures quite comparable to those obtained in the dark. The control glucose culture in this experiment grew much more slowly, however, than the control for the cultures in darkness, so that the parallelism of the cultures in the two experiments must be regarded as relative rather than absolute.

A measure of the average rate of growth in the fructose medium may be made by calculating the straight line of nearest fit to the logarithms of the average bulk in the two cultures. This is found to be  $y = 0.186x + 2.42$ , from which it appears that the average rate of growth in the fructose medium is about 73 per cent. of that in the glucose medium.

The interesting observation was made towards the end of the experiments, that in the fructose medium the alga had almost completely changed



TEXT-FIG. 11. Diagram to show relative rates of growth of *Scenedesmus* sp. in liquid media containing sucrose (cultures A and C) and glucose (culture B): for explanation see text.

its mode of division, as shown in Text-fig. 11. Mother-cells containing four or eight autospores were extremely rare, and the much-enlarged cells were observed to multiply by constriction and in a few cases by genuine budding, in a manner entirely foreign to the Autosporaceae, to which group the genus *Scenedesmus* belongs.

This feature was observed to a smaller extent in the cultures in glucose in the dark.

(iii) *Growth in a Medium containing Sucrose.*

Two cultures in sucrose were compared with one in glucose in dim light; the side tube of flask C was broken after six samples had been taken, and the culture had therefore to be discarded. The results are given in Table X and plotted in Text-fig. 11.

TABLE X.

*Bulk of Alga per c.mm. of Medium (log. values) in Cultures containing Sucrose and Glucose.*

<i>Day of Expt.</i>	<i>Culture A.</i>	<i>Sucrose.</i> <i>Culture C.</i>	<i>Glucose.</i> <i>Culture B.</i>
0	2.5042	2.5042	2.5042
1	2.6179	2.7004	2.8899
2	3.0305	3.1559	3.2645
3	3.2702	3.3224	3.3098
4	3.4642	3.4699	3.6682
5	3.6825	3.7603	3.8767
6	3.7986	—	4.3867
7	4.3872	—	4.5959
8	4.5607	—	4.9660
9	4.9508	—	5.4343

The straight lines calculated to fit the observed values for the three cultures are :

$$\left. \begin{aligned} y &= 0.268x + 2.42, \text{ for A} \\ y &= 0.250x + 2.526, \text{ for C} \end{aligned} \right\} \text{ sucrose,}$$

and  $y = 0.309x + 2.5$  for B, glucose.

The deviation of some of the observed values from the calculated lines is considerable, and the curves have therefore been examined statistically by the method used for the galactose cultures.

The standard error for the sixteen observations made on the two sucrose cultures is found to be 0.0555, while the difference in slope of the two calculated lines is 0.268 to 0.250, i. e. 0.018. The difference in slope of the lines is thus only one-third of the standard error, and the rates of growth in the two cultures are therefore not significantly different.

When the ten values for culture B, in glucose, are also included, the standard error for the whole series becomes 0.0157. The divergence between

the glucose line and the average of the two sucrose lines is  $0.309 - 0.259$ , i. e.  $0.050$ ; this is  $3.185$  times the standard error, and the rate of growth of the glucose culture is therefore significantly different from that of the two sucrose cultures.

The rate of growth of the alga in the sucrose medium is thus  $259/309$ , or about 84 per cent. of that in the glucose medium.

(iv) *Growth in a Medium containing Maltose.*

Three attempts have been made to study the growth of the alga in a solution containing maltose, but, owing to accidents of different kinds, it is impossible to place absolute reliance in any of the results; a detailed consideration of them is therefore deferred to a later date. It may be noted, however, that for a period of ten days, in two parallel cultures with maltose, the organism grew at identically the same rate and in the same manner. In contrast to the usual uniform rate of growth in a glucose medium, the curves showed a continuous increase in the growth rate for five days, after which the values for the two cultures lay approximately on a single straight line.

The cultures are thus extremely interesting in that they are the only ones in which the existence of an initial 'lag' period has been indubitably observed, such as has been so strongly emphasized in the case of cultures of bacteria and of protozoa. No such lag has ever been observed in a culture with glucose, and it may be suggested tentatively that its existence in the maltose medium may be due to the necessity for the production of maltase for the conversion of the maltose into glucose before it can be assimilated by the organism. Owing to the accidental destruction of the results of the control culture in glucose it is not quite certain, however, that this lag may not have been due to some unusual physiological condition of the mother culture which might have been reflected also in the growth of the control culture.

Some support is lent to the above suggestion by the fact that in a second pair of parallel cultures with maltose, both of which were observed after some days to be contaminated, the 'lag' was comparatively slight and extended for two days only, after which the rate of growth in the two cultures was almost identical with that in the control culture with glucose. The presence of a second organism, already provided with maltase and therefore capable of supplying an increasing amount of glucose to the alga, would very considerably affect the slope and duration of the lag.

The third experiment was inconclusive because the control culture, though it showed no lag period, exhibited deviations from the straight line sufficiently great to throw some suspicion on the physiological condition of the mother culture from which the inoculations were made.

(v) *Growth in a Medium containing Xylose.*

Two cultures, A and C, in a medium containing 1 per cent. xylose, were set up to compare with one glucose culture, B.

In culture C the numbers of cells counted in ten drops for the first six days were 13, 11, 9, 11, 9, and 7 respectively, and at the end of that time most of the cells had shrunken contents and looked unhealthy.

In culture A the numbers were 13, 15, 13, 38, 17, 21. The drawings of the cells indicate an increase in size up to the fourth day, when multiplication began to take place, after which the average diameter decreased considerably. From that time onwards there was no apparent growth in the culture. At the end of nine days there was still no significant increase in numbers, the cells were all of medium size, and there were no signs of division; a few of the larger cells had lost their colour. Three weeks later there were still a few green cells in the medium, but fewer than had been sown, and a number of colourless cells with shrunken contents. Evidently division had taken place to a very limited extent, followed by death of the young cells, and it is concluded that under the conditions of the experiment xylose is actually toxic to the species.

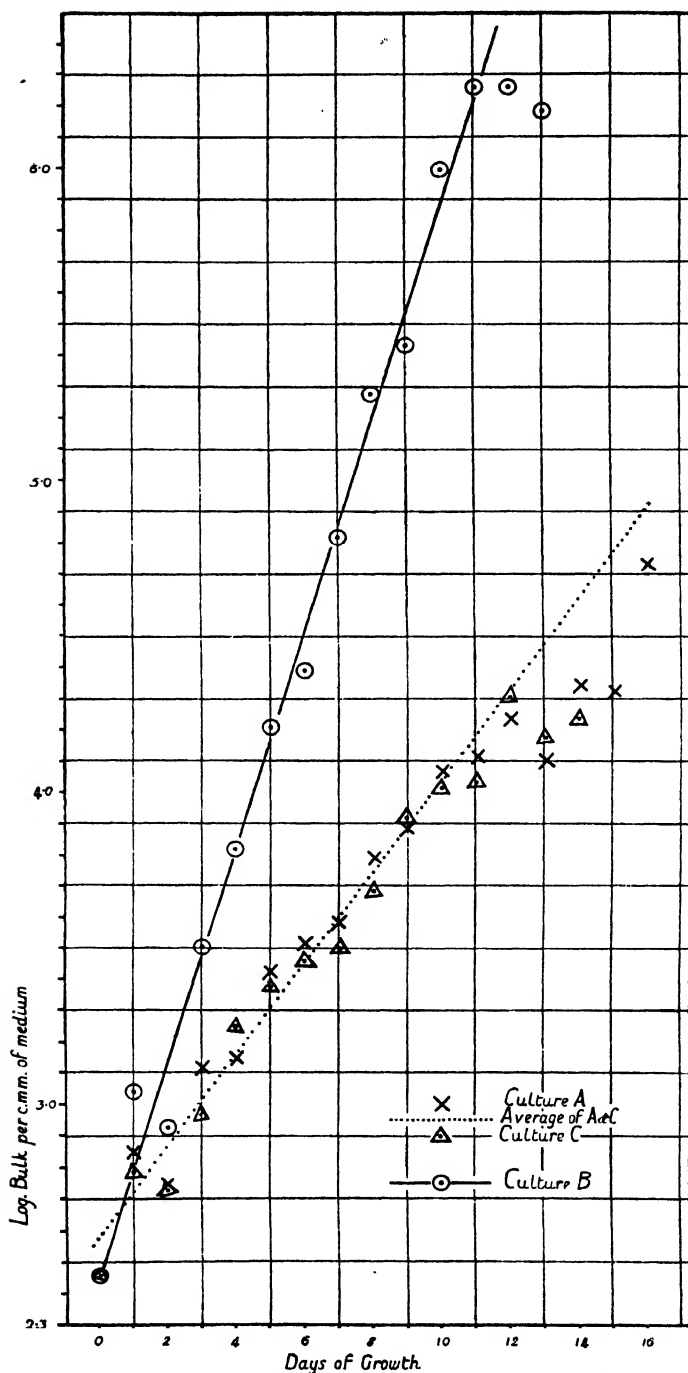
(vi) *Growth in a Medium containing Glycerine.*

Two cultures in a glycerine medium were compared with one in a glucose medium, the results being recorded in Table XI and plotted in Text-fig. 12. They were grown with constant illumination from an electric lamp.

TABLE XI.

*Bulk of Alga per c.mm. (log. values) in Media containing Glycerine and Glucose.*

<i>Day of Expt.</i>	<i>Culture A.</i>	<i>Glycerine.</i>	<i>Culture C.</i>	<i>Glucose.</i> <i>Culture B.</i>
0	2.4545		2.4545	2.4545
1	2.8477		2.7812	3.0399
2	2.7471		2.7281	2.9226
3	3.1149		2.9688	3.5040
4	3.1462		3.2507	3.8118
5	3.4216		3.3813	4.2100
6	3.5172		3.4596	4.3773
7	3.5813		3.5086	4.8141
8	3.7919		3.6892	5.2750
9	3.8833		3.9191	5.4302
10	4.0649		4.0174	5.9985
11	4.1134		4.0312	6.2563
12	4.2386		4.3103	6.2591
13	4.1038		4.1780	6.1830
14	4.3474		4.2442	—
15	4.3241		—	—
16	4.7313		—	—



TEXT-FIG. 12. Diagram to show relative rates of growth of *Scenedesmus* sp. in liquid media containing glycerine (cultures A and C) and glucose (culture B): for explanation see text.

There is little doubt from the figure that the straight line  $y = 0.343x + 2.455$ , calculated to fit most nearly the observed values, represents the rate of growth of the alga in the glucose medium. In the glycerine medium, however, there appear to be fairly regular fluctuations in rate of growth, and there is such a close agreement between the parallel cultures that it seems unlikely that such fluctuations are fortuitous. A measure of the average rate of growth of the alga in the glycerine medium may be made by calculating the straight line of nearest fit to the logarithms of the average bulk in the two cultures. The equation to this line is found to be  $y = 0.146x + 2.57$ , and it is seen from the diagram that from the sixth to the tenth days this line represents the observed values fairly closely.

The average rate of growth of the alga in the glycerine medium is thus  $0.146/0.343$ , or nearly 43 per cent. of that in the glucose medium.

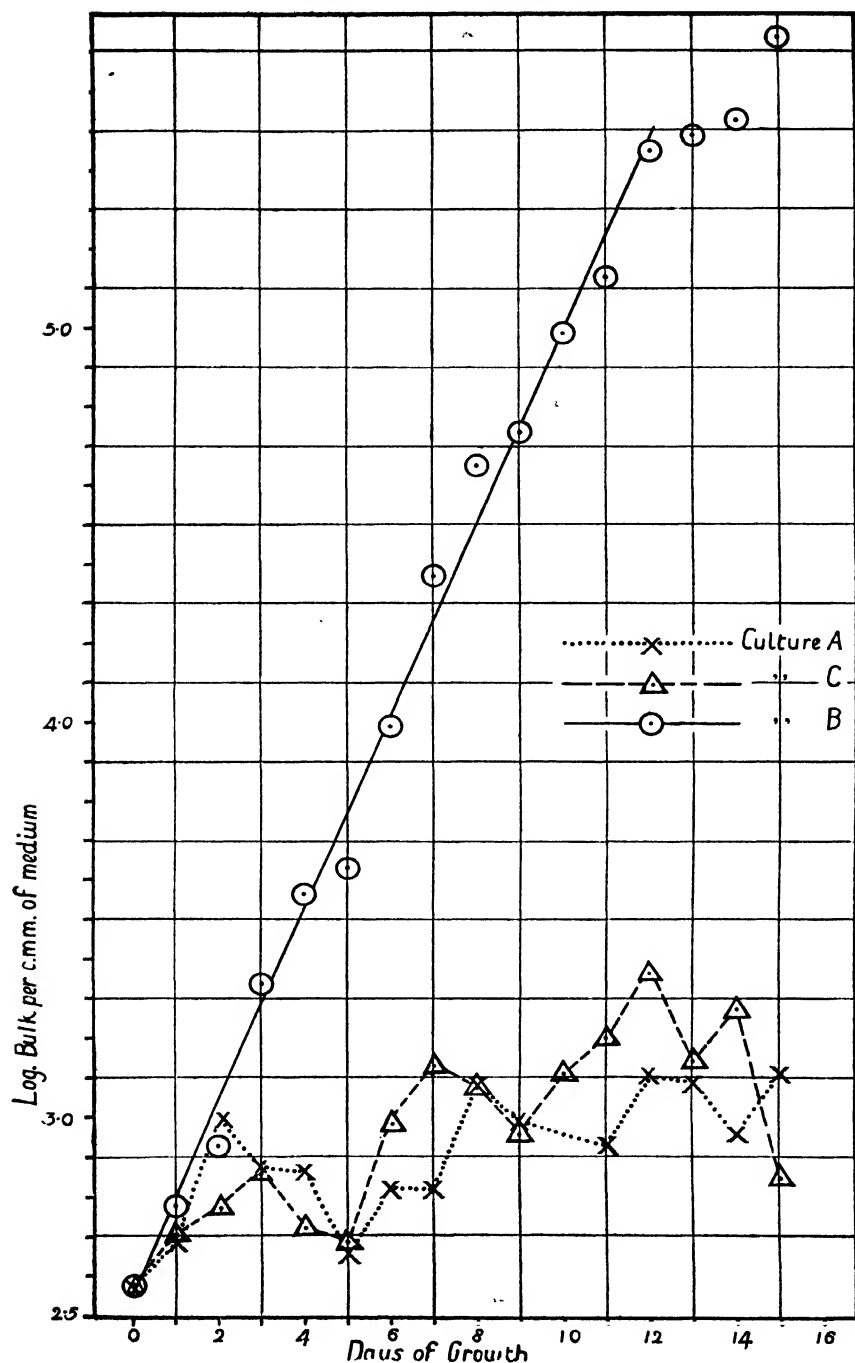
(vii) *Growth in a Medium containing Mannite.*

Mannite was included among the organic compounds to be investigated because it figures prominently as a source of carbon in a number of bacteriological media, in particular in those for the cultivation of *Azotobacter*. It had also been observed to promote vigorous growth in certain other algal species. Parallel cultures in a mannite medium were compared with one in

TABLE XII.  
*Cultures in Mannite and in Glucose.*

Day of Expt.	Mannite.			Glucose.	
	Average Diam. of Cells. Two Cultures.	Average No. of Cells per c.mm.	Log. Bulk.		Culture B.
			Culture A.	Culture C.	
(1)	(2)	(3)	(4)	(5)	(6)
0	8.1	1.3	2.5753	2.5753	2.5753
1	8.1	1.6	2.6891	2.7096	2.7773
2	7.9	2.7	2.9916	2.7753	2.9291
3	6.9	4	2.8723	2.8683	3.3377
4	6.1	4.5	2.8600	2.7220	3.5630
5	5.5	4.1	2.6529	2.6881	3.6316
6	6.5	4.8	2.8190	2.9908	3.9899
7	7	5.2	2.8191	3.1308	4.3686
8	7.2	5.5	3.0799	3.0762	4.6489
9	6.2	5.8	2.9872	2.9614	4.7337
10	6.6	6	—	3.1100	4.9861
11	6.7	7.3	2.9244	3.2052	5.1283
12	7.1	8.3	3.1031	3.3683	5.4455
13	6.5	8.1	3.0870	3.1432	5.4840
14	7	6.4	2.9567	3.2784	5.5281
15	7.2	4.5	3.1102	2.8463	5.7327
16	—	4.8*	—	—	—
21	—	3.5*	—	—	—
34	—	9.8*	—	—	—
37	—	7.3*	—	—	—
83	—	103 *	—	4.4972	—
116	—	260 *	—	4.8465	—

\* Culture C only.



TEXT-FIG. 13. Diagram to show relative rates of growth of *Scenedesmus* sp. in liquid media containing mannite (cultures A and C) and glucose (culture B): for explanation see text.



a glucose medium, the results being summarized in Table XII and plotted in Text-fig. 13.

The average rate of growth in the glucose culture is represented by the straight line  $y = 0.2434x + 2.55$ , seven of the eleven points from which it was calculated lying very close to the line.

The values for the mannite cultures, however, present quite a different aspect from any others that have been obtained. The bulk of alga is seen to rise to a maximum and then fall, rising to a second maximum a few days later and again decreasing, before rising to a third maximum, and so on. This fluctuation in the bulk of alga was observed to be caused by the division of the cells into autospores, of which a great number died; the survivors grew to a certain size and again divided, with the subsequent death of a considerable proportion of the young cells. This is brought out clearly by a comparison of columns (2) and (3) of Table XII. The average size of the cells decreased continuously until the seventh day, indicating an increasing proportion of small cells; the numbers, on the other hand, increased only very slowly, showing that many of the progeny of the mother-cells must have failed to grow, and in some cases the numbers themselves actually decreased. Disintegrating autospores were frequently met with in the material examined.

Successive maxima on the logarithmic curve are seen to become progressively higher, and when culture B was examined at the end of three months it contained 103 living cells per c.mm. of medium, with a logarithmic bulk of 4.4972; a month later the number of cells had increased to 260 per c.mm., with a corresponding bulk of 4.8465.

Since, in this species, no sexual fusion has been observed at any period of its life-history, this manner of growth cannot be explained satisfactorily on the basis of the separating out of a special strain of the organism from heterozygous material; it appears rather to be a case of the 'education' of the species to tolerate a medium which is not primarily suited to its growth, or of the breeding out of a physiological strain capable of using the substance supplied to it, or it may be that the organism gradually effects a change in the medium so that it becomes more favourable to its growth.

The average resultant rate of growth of the two mannite cultures for the first sixteen days may be found by calculating the straight line of nearest fit to the logarithms of the average bulk in the two cultures. This is found to be represented by the equation:

$$y = 0.032x + 2.7,$$

as against  $y = 0.2434x + 2.55$  for the control glucose culture;

i. e. the average rate of growth in the mannite medium is only  $0.032/0.2434$ , or about 13 per cent. of that in the glucose medium.

## D. GENERAL DISCUSSION OF THE EXPERIMENTAL RESULTS.

The foregoing experiments demonstrate a number of very interesting facts, among which the ability of the alga *Scenedesmus costulatus*, var. *chlorelloides*, to grow in the dark is highly significant, since it shows that this organism is capable of growing vegetatively in the lower layers of the soil, given a supply of suitable food, and that it is therefore a factor which must be taken into consideration in studying the biological and chemical changes which take place in the soil. Since this organism is only one of a number of species that have been isolated from the lower layers of the soil, it is probable that it is only one example of many algal species which can grow there saprophytically, and by the processes of their metabolism effect changes which may be reflected in the fertility of the soil. Preliminary experiments have shown that at least two other species isolated from the soil can develop in complete darkness.

Parallel cultures of the alga, inoculated with equal quantities of a uniform suspension of cells, and kept under the same conditions of temperature and light, are seen to increase in bulk in the same manner and at the same rate, within the limits of experimental error: no pair of parallel cultures has yet been observed which exhibited a significant difference in the rate of growth. Text-figs. 3, 6, 7, 8, 9, 11, 12, and 13 demonstrate the truth of this statement, which is very strongly supported by the statistical analyses of the results.

The size of the cells varies conspicuously from day to day, and the number of cells per unit volume of medium increases in a very erratic manner; but if the two sets of observations, number and size, are combined together in a single calculated estimation of the bulk of algal protoplasm, the logarithmic values of the bulk for about the first ten days of the experiment are found to lie, within the limits of experimental error, upon a straight line in those media completely favourable to the growth of the organism. This indicates that the organism is growing at a uniform rate in these favourable media.

Of the media examined, that containing mineral salts + 1 per cent. glucose seems to be most favourable to the growth of the organism, both in regard to the bulk of alga produced in a given time and to the degree of deviation of the observed points from the calculated straight line which most nearly fits them; this deviation is shown to be considerably reduced by continuous aeration of the culture (Text-fig. 4) and by control of the illumination (Text-fig. 5).

A greater degree of variance of the experimental values from the straight line of nearest fit to them has been observed in those media which are only moderately favourable to the growth of the organism. In some experiments this may have been partly due to slight variations in the light

intensity, but it is more likely to be the expression of the working of a physiologically unbalanced nutrient medium. In the media under consideration enough of the sugar was added in each case to give approximately equal numbers of carbon atoms per litre in the different media. In those containing hexoses, pentoses, and mannite this would entail only slight variations in the molecular concentration of the medium, but in those containing glycerine and the disaccharoses it would be necessary to add respectively twice as many and only half as many molecules of the organic compound to give the same number of carbon atoms per litre. This might cause slight irregularities in the physiological balance of the media apart from those due to the chemical nature of the compound under investigation, and might account to some extent for the greater degree of variance of the observed points.

In media containing fructose, glycerine, and mannite the logarithmic values of the bulk do not lie scattered indiscriminately about the calculated straight line of nearest fit, but fluctuate in a somewhat rhythmical manner, suggesting that the rate of growth is not really uniform during the period of observation. In the cultures with mannite this fluctuation is partly to be ascribed to the death of a proportion of the autospores after they have been set free from the mother-cells, but in the cultures with fructose and glycerine no such death was observed, and, if the fluctuations are more than accidental, they may possibly indicate that fructose and glycerine are not equally assimilable at all stages of development of the individual cells.

There is no satisfactory evidence of the existence of a definite 'lag period' in the growth of the organism after being inoculated into any of the media investigated, except in that containing maltose; in this medium, however, there has been observed a gradual increase in the rate of growth up to the seventh day of the experiment, after which the rate appeared to be uniform; and there is some reason to believe that the final rate of growth attained by the organism in the maltose medium is not less than that in the glucose medium. It is seen in Pl. VII, A, that the amount of growth in the maltose culture is not much less than that in the glucose culture, though appreciably greater than that in the galactose culture.

If the rate of growth of the organism in a glucose medium be taken as 100 per cent., then it is possible to give values to the other compounds examined as shown in Table XIII.

In addition to the information which these experiments provide in regard to the physiology of nutrition of the organism concerned, they offer a useful series of data which are of extreme interest from the general point of view of the physiology of growth. The fact that, when plotted against time, the logarithmic values of the bulk for about ten days lie upon a straight line, within the limits of experimental error, demonstrates that the relative rate of increase in bulk of the organism from day to day is

constant over this period; that is to say, the growth in bulk of *Scenedesmus costulatus*, var. *chlorelloides*, in certain liquid media may be expressed mathematically for a limited period of time by a simple exponential curve. The highly controversial nature of this subject makes it quite impossible to deal adequately with the experimental results from this point of view in the present paper; their discussion is therefore deferred to a later paper, in which their bearing on the results of investigators of other groups of unicellular organisms may be dealt with in greater detail.

TABLE XIII.

Description of Growth.	Organic Compound.	Growth Rate of the Organism, expressed as a Percentage of the Growth Rate in Glucose.
I. Vigorous normal growth at a uniform rate for about ten days	Glucose (in the light)	100 %.
	Maltose	100 % after lag period (†).
	Galactose	94 %.
	Sucrose	84 %.
II. Moderate growth at a less uniform rate	Fructose	73 %
	Glucose (in darkness)	40 %
	Mineral salts alone	60 %.
	Glycerine	43 %.
III. Retarded growth	Mannite	13 %.
IV. No growth	Xylose	0 %.

{ Cells much enlarged and often abnormal in division.

## SUMMARY.

*Part I.*

A method is described for obtaining pure cultures of algae from the soil.

In pure cultures of soil algae on solid media, the great majority of species show greatly increased growth in the presence of a number of different soluble organic compounds, each species having its own order of selection of the compounds that have been tested; a few species do not behave in this way, and are possibly completely autotrophic in nutrition.

Pure cultures of several soil species in liquid media containing glucose showed that the best estimate of the growth of a unicellular alga may be obtained by making daily measurements of the average size of the cells and of the number of cells per unit volume of liquid, and by calculating from these data the bulk of algal protoplasm present. The logarithmic values of the bulk when plotted against time lie upon a straight line within the limits of experimental error for a limited period of growth; the slope of this line (i. e. the tangent of the angle which it makes with the horizontal axis) may be taken as a measure of the rate of growth of the organism in the given medium.

## Part II.

A method is described by which the growth of the alga *Scenedesmus costulatus*, Chod., var. *chlorelloides*, nov. var., has been studied quantitatively in liquid media containing mineral salts + 1 per cent. of certain soluble organic compounds. In the glucose medium, the degree of variance of the observed values (logarithmic) from the calculated straight line of nearest fit is shown to be greatly reduced by rigorous control of light and of temperature, and by continuous aeration of the medium. In this medium the organism is able to grow in the dark, retaining its green colour. There is some reason to believe that the rate of growth in the dark may be approximately equal to the difference between its activities in the light in the same medium and in that with mineral salts alone. In certain media containing substances less favourable to the growth of the organism the degree of deviation of the observed values from the straight line is greater than in the glucose medium. With maltose there appears to be an initial 'lag' period preceding the straight-line period of growth. In mannite there are conspicuous fluctuations in the growth rate due to death of the young cells. Xylose is completely toxic to the organism under the conditions observed.

The relative average rates of growth in the different media may be expressed quantitatively, as follows: Glucose in the light 100 per cent., maltose 100 per cent. preceded by a 'lag' period (?), galactose 94 per cent., sucrose 84 per cent., fructose 73 per cent., mineral salts alone 60 per cent., glycerine 43 per cent., glucose in darkness 40 per cent. (?), mannite 13 per cent., xylose 0 per cent.

The data provide evidence of the fact that, in those media that are completely favourable to its growth, the increase in bulk of *Scenedesmus costulatus*, Chod., var. *chlorelloides*, follows the same laws as a simple exponential curve, for a limited period of time.

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## EXPLANATION OF PLATE VII.

Illustrating Dr. B. Muriel Bristol Roach's paper on the Relation of Certain Soil Algae to some Soluble Carbon Compounds.

Photograph to illustrate difference in growth of two species of soil algae on media containing different soluble carbon compounds.

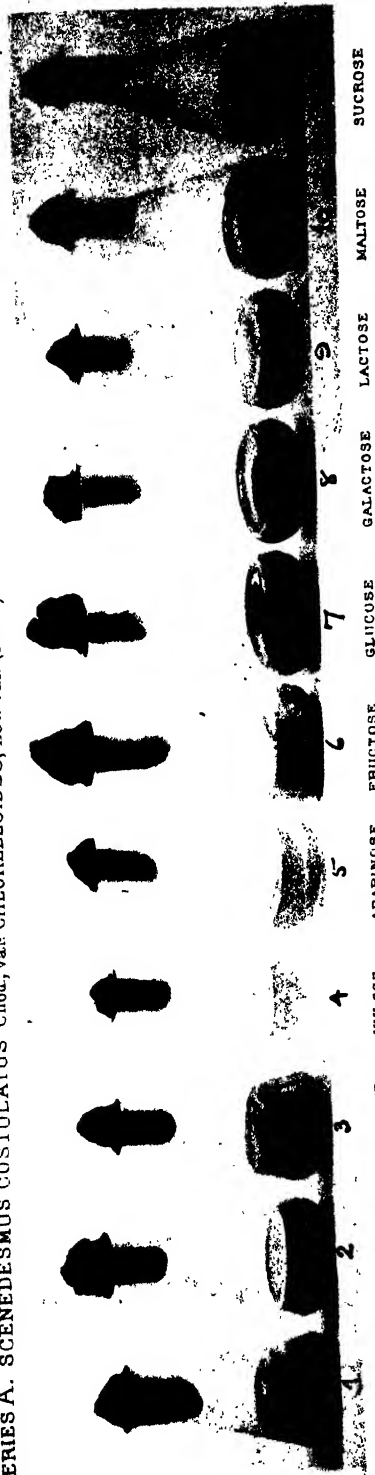
Series A. *Scenedesmus costulatus*, Chod., var. *chlorelloides*.

Series B. *Cystococcus* sp.

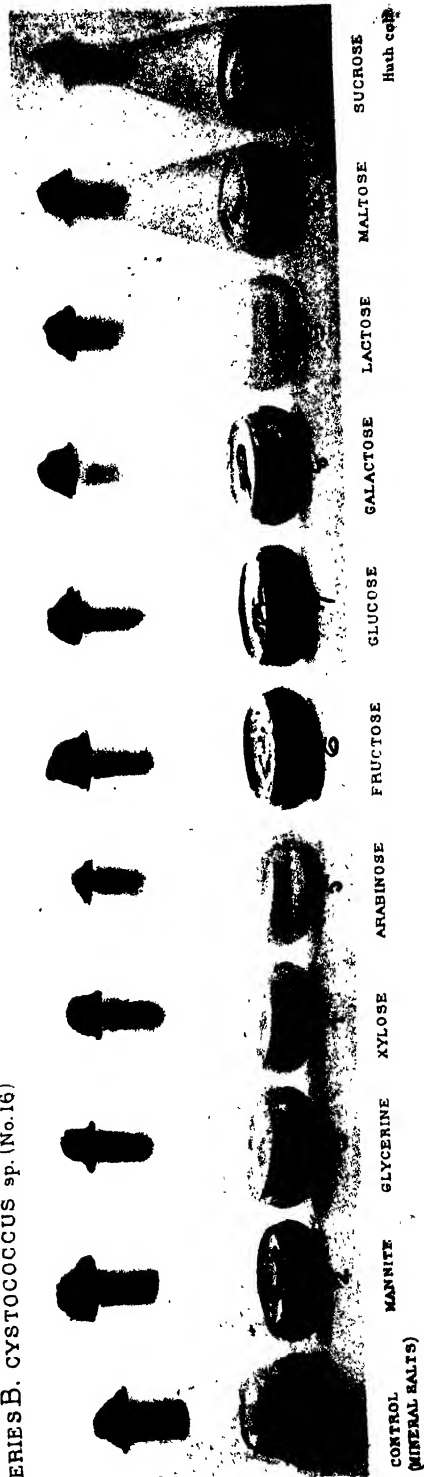


*Annals of Botany,*

SERIES A. SCENEDESMUS COSTULATUS Chod., var. CHLORELLOIDES, nov. var. (No.11)



SERIES B. CYSTOCOCCUS sp. (No.16)







# Studies in the Genus *Fusarium*.

## III. An Analysis of Factors which determine Certain Microscopic Features of *Fusarium* Strains.

BY

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AND

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With Plate VIII and three Figures in the Text.

IN the preceding paper of this series<sup>1</sup> an account was given of the manner in which the macroscopic features of certain *Fusarium* strains respond to cultural conditions. The present paper is complementary to the preceding one and deals with the variation of certain microscopic features under the same circumstances. The observations carried out in this connexion have centred almost entirely round the spores, and deal with such characters as the number of the septa, spore length width and shape, the appearance of the contents, the presence or absence of constrictions at the septa, &c. Particular attention has been paid to the degree of the spore septation, as this lends itself easily to quantitative treatment.

The general methods of culture have been explained in the previous paper and need not be repeated here. There remains merely to describe the method which was found to be suitable for microscopic study of the spores, especially as regards the number of septa.

According to the cultural treatment the spores of any strain may show hyaline, granular, or vacuolated contents. In the first case the septa show up perfectly clearly when the spores are mounted simply in water. With the granular type of spore, the dense nature of the cell contents in many cases is responsible for completely obscuring the septa, so that staining is in general necessary. For this purpose an aqueous solution of ruthenium red has proved to be of great value, the spores being mounted directly in

<sup>1</sup> Ann. Bot., xxxix, pp. 373-408, 1925.

a drop of the stain. Staining is almost instantaneous and is practically confined to the spore walls and the septa. In general it can be said that, the more granular the spore contents are, the greater is the difficulty in staining the septa clearly, but this difficulty has always been overcome by suitably increasing the concentration of the stain. Spores with vacuolated contents in some cases prove troublesome, as the line of junction of two vacuoles in the same segment is not always clearly distinguishable from a septum. Staining is again an advantage, but in spite of this and high magnification some difficulty has been experienced from time to time with spores of this type. Vacuolation is a feature which increases with the age of the spores and is followed in due course by the death of segments of the spore, this feature in turn becoming more pronounced as time goes on. All these degenerative features make the counting of the septa difficult, so that it is advisable to carry out the observations before these changes have gone too far. In practice a period of about fourteen days from the time of inoculating the plates was found to be suitable in this respect.

In some cases practically all the spores in a sample may have the same septation, e.g. they may all be 3-septate with only an occasional spore of different septation, in which case a process of counting is unnecessary. When, however, the sample is heterogeneous as regards degree of septation, recourse must be had to counting. After a number of trials it was decided to take counts of fifty spores as giving reasonable accuracy without prohibitive labour. The following table shows the result of ten successive counts of fifty spores from a sample consisting of a mixed lot of 3-, 4-, and 5-septate spores:

TABLE I.

	3-septate.	4-septate.	5-septate.	Average Septation.
1st 50	14	16	20	4.12
2nd "	20	12	18	3.96
3rd "	18	19	13	3.90
4th "	18	14	18	4.00
5th "	17	16	17	4.00
6th "	20	14	16	3.92
7th "	19	19	12	3.86
8th "	18	12	20	4.04
9th "	24	12	14	3.80
10th "	20	10	20	4.00
Average	19	14	17	3.96

The average of the whole 500 spores counted would be described as a fairly even admixture of 3-, 4-, and 5-septate spores, and the same description would be given to each one of the individual counts of fifty. Hence it appears that one count of fifty spores is sufficient to give a general idea of the kind of septation shown by any particular sample of spores, and this conclusion has been borne out by the general experience gained in the course of the present work. The conclusions put forward in this paper are based

on upwards of 600 counts of fifty spores each, together with about twice that number of observations in which the nature of the septation was recorded from general impression without actual counting.

In the last column of the above table is given the average number of septa per spore. This average number is useful for the rough graphical representation of the degree of septation, though it is clear that it only partially represents the result as given in the count.

Before proceeding to put forward a rule which gives the clue to the variation of septation in these *Fusarium* strains, it is advisable first of all to mention certain points by way of clearing the ground. These refer to the method of taking spore samples. Practically all the cultures studied have been cultures in Petri dishes, inoculated at the centre and allowed to grow out in circular form. In many cases spore samples could be obtained at any part of the colony, but from experience it was found that the best place to take the spore sample from was a short distance, say  $\frac{1}{2}$  to 1 cm., from the centre. The reasons for this are as follows. Whereas in some cases the degree of septation is practically the same over the whole plate, so that it is immaterial from which part the sample be taken, in other cases, as will be shown later, this is not so. Thus for comparison of strain with strain some kind of standard is advisable, and as it happens that some feebly sporring strains only sporulate in the neighbourhood of the centre of the colony, it follows that samples taken from the neighbourhood of the centre are most suitable for general comparative purposes. Thus, unless the position is specified otherwise, the data relative to septation refer to the spore masses at a short distance from the centre.<sup>1</sup>

Again, there is definite evidence that the degree of septation at any one spot increases for some time after the spores have formed, i. e. that the spores have not yet developed their full complement of septa. This feature has also been noted by other workers on *Fusarium*.<sup>2</sup> The following figures (Table II), representing counts of fifty spores taken at different dates from the same ring of spores (strain A), illustrate this point. The ages of the spores as put down in this table are upper limits; thus the spores described as being three days old are actually somewhat younger, as they were formed at a region of the culture which coincided with the growing margin three days earlier.

<sup>1</sup> The absolute centre of the colony should be avoided, as there is definite evidence that the septation there is often lower than elsewhere. When the inoculum has been a piece of either mycelium or substratum from the parent culture, it very frequently happens that the new colony proceeds at a very early stage to form spores *in* the inoculum. Cases have been seen where the piece of mycelium or substratum, on transference to a new medium, began forming spores almost immediately, a certain proportion of these spores then germinating *in situ*. These spores, as has been stated, are generally subnormal in their degree of septation.

<sup>2</sup> e. g. Appel and Wollenweber, Arb. a. d. kais. biol. Anst. f. Land- u. Forstwirtschaft, viii, p. 1-207, 1913.

TABLE II.

Age.	0-septate.	1-septate.	2-septate.	3-septate.	4-septate.	5-septate.	Average
3 days	2	1	0	9	25	13	3.86
	1	1	0	10	26	12	3.90
	1	1	0	7	26	15	4.02
	2	2	0	13	18	15	3.76
5 "	0	0	0	4	17	29	4.5
	0	1	0	9	18	22	4.2
7 "	0	0	0	8	17	25	4.34
	0	0	0	4	23	23	4.38
12 "	0	0	0	4	14	32	4.56
	0	0	0	3	15	32	4.58

The increase in septation is distinct between the third and fifth days, and is shown chiefly in the increase in number of 5-septate spores and in the disappearance of those spores showing 0- and 1-septation. The latter are of the length associated with 3- or 5-septate spores, that is, they are not the short type of spore which always remains of low septation, and it is clear that such spores are in process of forming septa. Hence it is necessary in making counts of septation not to use too young spores. At the same time it is advisable not to use spores which are too old, otherwise the vacuolation and atrophy incidental to age will in many cases make the counting troublesome. As a general rule the septation counts were carried out on cultures about two weeks old.

In the growth of the strains under consideration, the mode of septation is usually 3, 4, or 5. On a given medium a particular strain may show a high 3-mode, while another strain under the same conditions shows a high 5-mode. Nevertheless, by a suitable choice of conditions, the 3-mode strain will develop a 5-mode, and vice versa. Furthermore, it is possible so to choose the composition of the nutrient medium that the septation mode is lowered to 0 or 1, and conversely a medium can be found on which the mode, at least of some of the strains, rises to 6 or 7. By variations in the composition of the medium such as will be described below, spores of all degrees of septation from 0 to 10 have been produced. The highest septation recorded in the course of this work was 14, but that was observed only once.

The variation in the degree of septation of these spores follows a very definite rule which with one or two minor corrections has been found to apply to all the results obtained in these counts of septation. For the sake of clearness, the general rule will be given here. It is that there is a strong correlation between the degree of septation of the spores and the intensity of staling shown by the colony itself. With minimal staling conditions, the septation of the spores is high; when strong staling is shown, the septation mode is low. Thus strains which grow in the unstaled manner until the whole plate is covered show a 5-mode (or higher); those which become staled and therefore form colonies of limited size show spores with a septa-

tion mode of 3 or less. The rule applies both as regards different strains on the same medium and the same strain on different media. The application of this rule in general and the modifications required in certain cases will be illustrated in the course of the present paper.

It has been shown, when dealing with the macroscopic features of these fungi, that the degree of staling shown by any particular strain becomes less as the concentration of the nutrient is diminished. Corresponding to this effect one finds that the degree of septation of the spores increases with dilution of the medium. Text-fig. 1 illustrates this correlation. Various concentrations of the standard synthetic medium (represented here by N) were used as media, viz. 2 N, 3 N/2, N, N/2, N/3, N/4, N/5. These concentrations are represented arbitrarily at equal distances along the  $x$ -axis. The continuous curves represent the diameters of the colonies on the various media after twelve days' growth. The dotted lines represent the average septation as derived from a count of fifty spores taken from each culture at a distance of 1 cm. from the centre of the colony. The curves for diameter and average septation are given for three strains, A, D, and F. Table III gives the more detailed figures for the septation of strain A.

TABLE III.

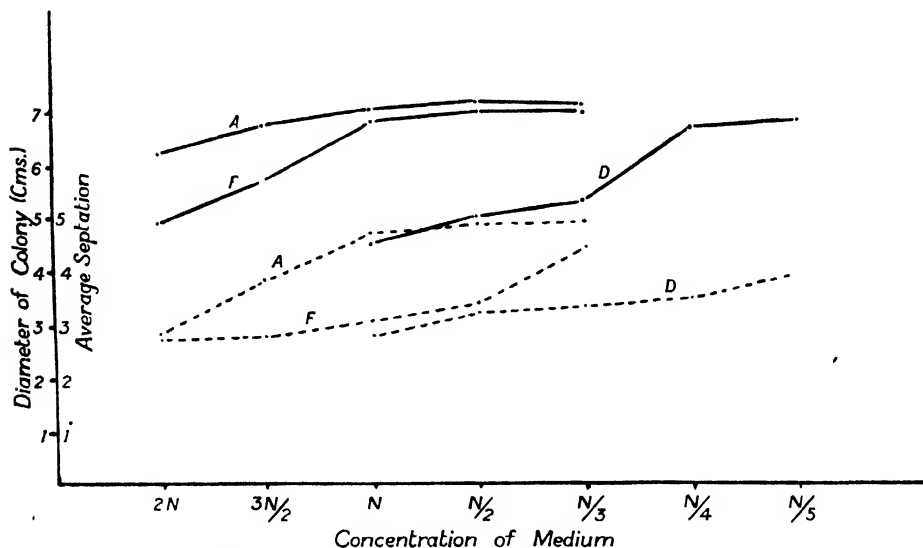
*Strain A.*

Medium.	1-septate.	2-septate.	3-septate.	4-septate.	5-septate.	6-septate.	Average.
2N	3	0	47	0	0	0	2.88
3N/2	0	0	21	13	16	0	3.90
N	0	0	1	11	37	1	4.76
N/2	0	0	0	3	47	0	4.94
N/3	0	0	1	4	44	1	4.90

Considering first the case of the two curves for one particular strain, say strain F, we see that they both rise from the left to the right. At concentrations 2 N and 3 N/2 there is distinct staling of the margin of the colony, and for both these media the average septation is low, corresponding to a high 3-mode. At the other end of the curve, with concentration N/3, staling effects are not shown, and here one finds the average septation has risen to 4.48, which represents a fairly high 5-mode. With the removal of staling, therefore, by dilution of the medium, the septation mode has been raised from 3 to 5. It will be noticed, however, that the rise in average septation got by dilution of the medium lags behind the rise in colony growth which is similarly produced. Thus at the concentration N/2 there is no marginal staling obvious, but still the average septation is comparatively low. The curves for strains A and D show the same features. The former has the least staling capacity of the three under consideration, and the graph shows that its spores have the maximum tendency to high septation. Thus even at the concentration N, at which both

the other strains show a high 3-mode, strain A shows a fairly high 5-mode. Similarly strain D is characterized by a maximum staling capacity and by a minimum tendency to high septation.

The dependence of degree of septation on concentration of medium is illustrated in the case of strains A, F, and D in Text-fig. 2,<sup>1</sup> which gives a series of tracings of representative groups of spores taken from photographs. It will be observed that in the case of each strain the degree of septation (and length) of the spores increases with dilution of the medium.



TEXT-FIG. 1. Illustrating the correlation between high spore septation with the non-staling type of colony growth. The continuous curves represent colony growth; the dotted curves represent average degree of spore septation on the various concentrations of medium.

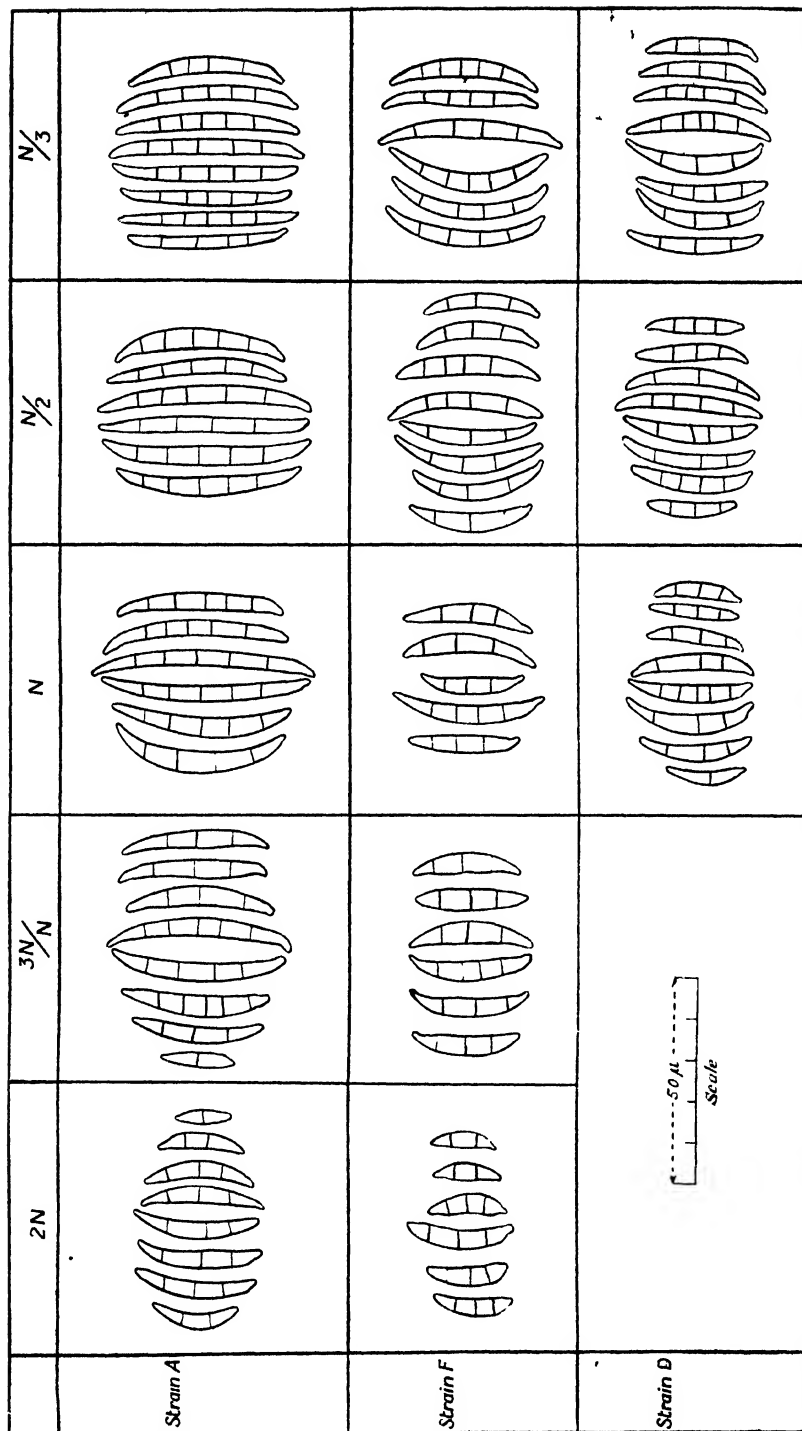
The spores of strain A already show a 5-mode when the stage of dilution represented by N has been reached, while at that concentration of medium strains F and D show 3-modes. At dilution N/3 strain F has reached a 4-mode, while strain D still shows a 3-mode, though there is a tendency for an increase in the number of spores of septation higher than 3.

Figs. 1 to 4 of Pl. VIII are photographs showing typical spores of strain A on media of concentration 4 N, 3 N, 2 N, and N respectively, where N is the standard synthetic medium.

The rule here indicated has been found to apply to all the strains (about fifty) of this *Fusarium* in culture, so much so that, when a new strain is grown on the standard medium, the septation of its spores can be foretold by observing the manner in which the colony grows. Table IV gives the

<sup>1</sup> We are indebted to Miss M. Reeks, Technical Artist in the Imperial College, for the drawings from photographs which have been used in the preparation of Text-figs. 2 and 3.

Concentration of Medium



TEXT-FIG. 2. Illustrating effect of concentration of medium on length of spore and degree of septation. Spores of strains A, F, and D on media of strength  $\frac{2}{3}N$ ,  $\frac{3}{2}N$ ,  $N$ ,  $\frac{N}{2}$ , and  $\frac{N}{3}$ , where  $N$  is the standard synthetic medium.



general behaviour of the four types into which it is proposed to group the members of this species. The growth features and the septation of the spores refer to colonies on the standard synthetic medium at laboratory temperature (*c.* 17°).

TABLE IV.

<i>Classification.</i>	<i>Growth.</i>	<i>Septation.</i>
Pionnotal type	staled	3-mode.
Sporodochial „	{ staled unstaled	3-mode. 5-mode, or mixture of 3-, 4-, and 5-septate spores.
Mycelial „	staled	3-mode.
Long spore „	unstaled	5-mode or higher.

Figs. 5, 6, 7 of Plate VIII are photographs of spores of strains D (mycelial type), A (weakly staling sporodochial type), and D<sub>1</sub> (long spore type) on the standard synthetic medium (with 1 per cent. potato starch added). The preponderance of 3-septate spores in strain D, of 5-septate spores in strain A, and the presence of a considerable proportion of spores with septation greater than 5 in strain D<sub>1</sub> are to be noted.

Attention was drawn above to the fact that, as the concentration of the nutrient medium is diminished, the rise in the growth curve takes place before the corresponding rise in the curve of average septation. Thus within a certain region of concentration of the medium the unstaled type of growth coexists with a condition of low septation in the spores. At first glance this would appear to offer an exception to the rule stated above, but a little consideration will show that this result is just what might be expected. Spore formation takes place some distance behind the growing apex, and obviously under more severe conditions as regards staling than obtain at the growing margin. Thus, on the hypothesis of the correlation of staling with low septation, one would anticipate that there would be a transitional region of concentration in which staling was not shown at the growing margin, but was shown by the lowered septation of the spores which are formed some distance back from the growing margin. This is what is found in actual practice, and is the lag effect above referred to.

Two further adjustments of the general rule may be given here. In Table IV it is pointed out that the pionnotal strains all show the staled type of growth on the standard medium, and that all have 3-septate spore modes. The same statements apply to such a mycelial strain as strain D. Nevertheless, one finds a larger number of spores with septation greater than 3 in the cultures of the pionnotal strain than in cultures of strain D, that is, while the average septation is low in both cases, it is lower for strain D than for a pionnotal strain which may show staling features quite as strong as does strain D. The difference is undoubtedly correlated

with the fact that the pionnotal strains begin to form spores much sooner, and therefore under conditions of less severe staling than in the case of a mycelial strain like D.

The same considerations will serve also to explain the following. It was shown in the preceding paper of this series that the curve of growth obtained on increasing the concentration of the medium fell to a minimum, after which it rose again, this latter rise being more pronounced on some media than on others. This rise is in some cases not accompanied by any appreciable rise in average spore septation. It must be remembered, however, that the salient feature of the colonies formed at these high concentrations is the increased permanence of the mycelium and the consequent late appearance of sporulation. The analogy between this case and those already considered is obvious.

With the corrections above noted, and with another to be mentioned later, the rule that high septation is correlated with reduced staling capacity, and conversely, has furnished the clue to the understanding of all the phenomena of spore septation observed throughout an extensive study of these *Fusarium* strains on a large variety of media.

The intensity of staling in any culture tends to increase as the colony grows, and hence it is that one finds a general tendency for the degree of septation to diminish towards the edge of the culture. Table V will serve to illustrate this point. Strain A was grown on a basal medium composed of

K <sub>3</sub> PO <sub>4</sub>	0.125 per cent.
MgSO <sub>4</sub>	0.75 „
Potato starch	2 „

with the percentages of asparagin shown in the table. The septation counts were carried out when the cultures were 22 to 24 days old. The last column in the table gives the diameter of the colonies (average of 3) after 15 days; the other figures give the average septation per spore, based as usual on counts of 50, and refer to samples taken at different distances from the centre. The septation counts for the media with asparagin, 0.02 per cent. to 0.2 per cent. inclusive, which are the critical media from the point of view in question, are the mean of two counts of 50.

On the first four media the degree of septation is sensibly uniform at different parts of the culture.<sup>1</sup> In the case of the medium with 0.05 per cent. asparagin, however, a very significant drop in the average septation occurs between radii 2 and 3 cm., and a similar effect is shown on the remaining media. On proceeding from media of low to those of high asparagin content one sees that the first indication of marginal staling

<sup>1</sup> On the first two media, which are starvation media so far as nitrogen is concerned, sporulation is feeble and confined to the neighbourhood of the centre of the colonies.

occurs on the medium with 0.2 per cent. asparagin, whereas the staling effect as reflected in the lowering of spore septation is already clearly shown in the marginal region of the cultures with 0.05 per cent. and 0.1 per cent. asparagin. This is the same lag effect as was alluded to in connexion with Text-fig. 1.

TABLE V.

% Asparagin.	Near Centre.	Position from which Spores were taken.				Average Diameter.
		Rad. 1 cm.	Rad. 2 cm.	Rad. 3 cm.	Rad. 4 cm.	
0	5.74	—	—	—	—	8.4
0.005	5.54	5.40	—	—	—	8.5
0.01	5.24	5.24	5.26	5.10	—	8.5
0.02	4.68	4.89	4.89	4.47	4.41	8.45
0.05	4.88	4.75	4.80	3.95	3.73	8.5
0.1	4.77	4.65	4.30	3.27	3.44	8.53
0.2	4.76	4.42	4.67	3.24	3.17	7.73
0.3	4.04	3.52	2.52	2.38	—	6.43
0.4	3.32	—	—	2.36	—	6.17
0.5	3.06	—	2.22	—	—	5.68

The above table illustrates the necessity, especially on certain media, of determining the degree of septation in a standardized manner if any value is to be placed on the figure obtained.

A repeat count carried out three weeks later gave substantially the same result, so that the tendency to reduced septation towards the margin of the culture was not an ephemeral effect due to the spores towards the margin being too young at the date of counting.

By way of further illustrating the correlation between staling of marginal growth and the degree of spore septation, the effect of certain external factors on both will now be described.

*Effect of Change of Temperature.* It was pointed out in the preceding paper that rise of temperature increased the tendency to staling. Similarly one finds that a rise of temperature tends to lower the degree of septation.<sup>1</sup> The effect is most strikingly shown when one chooses the particular strain and medium so that staling is just not shown at a given temperature and compares the degree of septation at this temperature with that at a higher one. Such a case is the following. Strain A on the standard medium to which 1 per cent. potato starch is added does not stale appreciably at 15°, but does so distinctly at 20°. Table VI gives the results of two counts of fifty spores for each temperature.

*Effect of Varied Phosphate.* The degree of staling in a culture can be modified to a certain extent by variation of the phosphate content, viz. decreased phosphate intensifies staling, and conversely. Similarly one finds that decreased phosphate tends to lower septation. The following figures

<sup>1</sup> See Johann, *Phytopath.*, xiii, p. 51, 1923; also Appel and Wollenweber, loc. cit.

TABLE VI.

		Number of Spores which are—					Average Number of Septa per Spore.
		1-septate.	2-septate.	3-septate.	4-septate.	5-septate.	
At 15°	0	0	7	6	37	4·60	
„ „	0	0	1	11	38	4·74	
At 20°	1	1	46	0	2	3·02	
„ „	0	0	48	2	0	3·04	

illustrate this point. Strain A was grown at 15° on two modifications of the standard medium—

I containing 0·5 per cent.  $K_3PO_4$ ,  
 II „ 0·01 „ „

the other constituents being as usual. Four counts of fifty spores in each case gave the following averages :

Medium I 3·86, 4·04, 4·18, 4·30. Av. 4·1.  
 „ II 3·34, 3·52, 3·54, 3·58. Av. 3·5.

Reduction of the concentration of phosphate is effective in lowering the septation of a comparatively feebly staling strain such as strain A. On the other hand, increased phosphate has scarcely any significant effect in raising the septation of a strongly staling strain.

The correlation of the staling type of growth with low average septation of the spores indicates that the growth-retarding substances formed by the fungus itself lower the average spore septation. One would anticipate, therefore, that the addition of growth-retarding substances to the original culture medium would have the same effect. The two following sections will show that this is the case.

*Effect of Acidity or Alkalinity of the Medium.* Table VII gives the diameters of colonies of strain A after seven and fourteen days on a series of media of varied original acidity or alkalinity, together with the average septation of spores taken from near the centre of the various colonies. The standard synthetic medium was used, and the different degrees of acidity and alkalinity were got by the addition of the appropriate amounts of malic acid and sodium carbonate respectively. The cultures were grown in the light at laboratory temperature, which, in the present case, was somewhat low (12–15°). The figures for average spore septation given in the last column are based on counts of 100 spores in each case.

This table brings out the general correlation of growth rate with degree of spore septation. It also indicates a tendency for the optimum for growth to move towards the acid end of the series as time goes on, and there is a further slight indication that the optimum for high septation lies

still nearer the acid end of the series than that for rate of colony growth. The explanation of these results is simple. The progress of growth in these colonies leads to the formation of alkali, and therefore, while a retarding concentration of acid may be present in the earlier phases of growth, it tends to disappear as time goes on. The same consideration will show that a growth-retarding concentration of acid may be present at the margin of the colony, while approximately neutral conditions may obtain nearer the centre, where spore formation is taking place, and thus on theoretical grounds one would expect that the optimum for high septation would be at a higher acid (original) concentration than that for growth. This effect is much more pronounced with some strains than with others; for example, with strain D the average septation continues to increase well beyond the region where diminished marginal growth sets in. This feature is no doubt to be correlated with the fact that strain D forms its spores comparatively late, and, though the marginal hyphae are growing in a retarding concentration of acid, the spores do not form until the reaction in the central region of the colony is much nearer neutral, or, in fact, slightly alkaline.

TABLE VII.

<i>Medium.</i>		<i>Diameters of Colonies.</i>		<i>Average Septation.</i>
		<i>After 7 Days.</i>	<i>After 14 Days.</i>	
Acid 1	% malic acid	0.4	1.1	2.01
"	0.5 % "	0.6	2.3	3.62
"	0.2 % "	1.9	6.1	4.45
"	0.1 % "	2.6	6.5	4.48
"	0.05 % "	2.9	6.6	4.60
Neutral		2.9	6.6	4.21
Alkaline	0.02 % $\text{Na}_2\text{CO}_3$	2.85	5.9	4.16
"	0.05 % "	2.5	4.8	4.23
"	0.1 % "	1.5	3.3	3.93
"	0.2 % "	0.9	2.8	3.60
"	0.5 % "	0.5	1.25	3.22
"	1 % "	0.25	0.7	2.91

*Effect of a Toxic Substance.* The addition of small doses of a toxic substance diminishes the rate of growth of the fungal hyphae, and in tests with strain A this diminished rate of growth was found to be associated with reduced spore septation. Table VIII refers to tests in which varying amounts of phenol were added to the standard medium.

*Effect of changing the C : N Ratio of the Medium.* This is by far the most effective way of changing the characters of the spores, both as regards their degree of septation and also in other respects, such as the appearance of their contents. A high C : N ratio tends to increase, a low C : N ratio to diminish, the average septation. When the ratio in question is increased by raising the C content of the medium, the tendency of the high C : N

ratio to increase septation is counteracted to some extent by the tendency of increased total concentration to lessen it, and in consequence the resultant effect on septation is not so striking, and in some cases is not demonstrable at all. When, however, the increased C : N ratio is obtained by diminishing the N content of the medium, the favouring effects of high C : N ratio and dilution of the medium reinforce each other, and very pronounced effects are obtained. The figures in Table V already quoted in another connexion illustrate this point clearly. A study of the first column of septation counts shows how the average septation steadily decreases from 5·74 to 3·06 as the asparagin concentration is raised from zero to 0·5 per cent. Table IX shows how, with diminishing C : N ratio, spores of high give place to spores of low septation. The figures given here are the actual counts from which the averages given in the first column of Table V are calculated.

TABLE VIII.

% Phenol.	Diameter of Colonies after 7 Days at 16°.	Average Septation.
0·0	3·6	4·06
0·01	3·2	3·38
0·02	1·3	3·1
0·04	0·5	No spores formed.

TABLE IX.

Concentration of Asparagin.	Number of Spores out of total of fifty which are—						
	1-septate.	2-septate.	3-septate.	4-septate.	5-septate.	6-septate.	7-septate.
0	0	0	1	3	13	24	9
0·005 %	0	0	1	2	19	25	3
0·01 %	0	0	0	3	34	11	2
0·02 %	0	0	6	11	27	6	0
0·05 %	0	0	2	4	42	2	0
0·1 %	0	0	4	6	38	2	0
0·2 %	0	0	3	8	37	2	0
0·3 %	0	0	12	24	14	0	0
0·4 %	0	2	33	14	2	0	0
0·5 %	1	0	44	5	0	0	0

Text-fig. 3 shows the variation of spore length and spore septation obtained in the case of the four strains A, F, C<sub>3</sub>, and D<sub>1</sub> by varying the asparagin content of the medium. The non-nitrogenous constituents in all cases were as in the standard synthetic medium, with the addition of 1 per cent. potato starch. The asparagin concentrations in the media from which the spores marked  $\alpha$  and  $\beta$  were derived were 0·5 per cent. and 0·01 per cent. respectively.

When the asparagin concentration is still further increased, say to 1 per cent. or 2 per cent., the spores are chiefly 0- and 1-septate, and the

mode is 1. Conversely, at the other end of the series, the highest mode that has been observed in any case (strain  $D_1$ ) was 7, the average septation being 6.62. Whereas it is not possible to raise the septation mode to 5 in the case of some strongly staling strains by mere dilution of the medium, this result is readily achieved by diminishing its N content. Fig. 8 of Plate VIII refers to the strongly staling strain D. The high 5-mode here shown is produced by reduction of the asparagin content of the standard synthetic medium.

The same type of result is obtained when the source of nitrogen is potassium nitrate or ammonium chloride. For equivalent concentrations effects very similar in degree are obtained with ammonium chloride as with asparagin; with potassium nitrate the effect is more gradual.

Figs. 12–16 of Plate VIII, which refer to strain A, illustrate the effect of increasing the C:N ratio on the degree of septation of the spores.

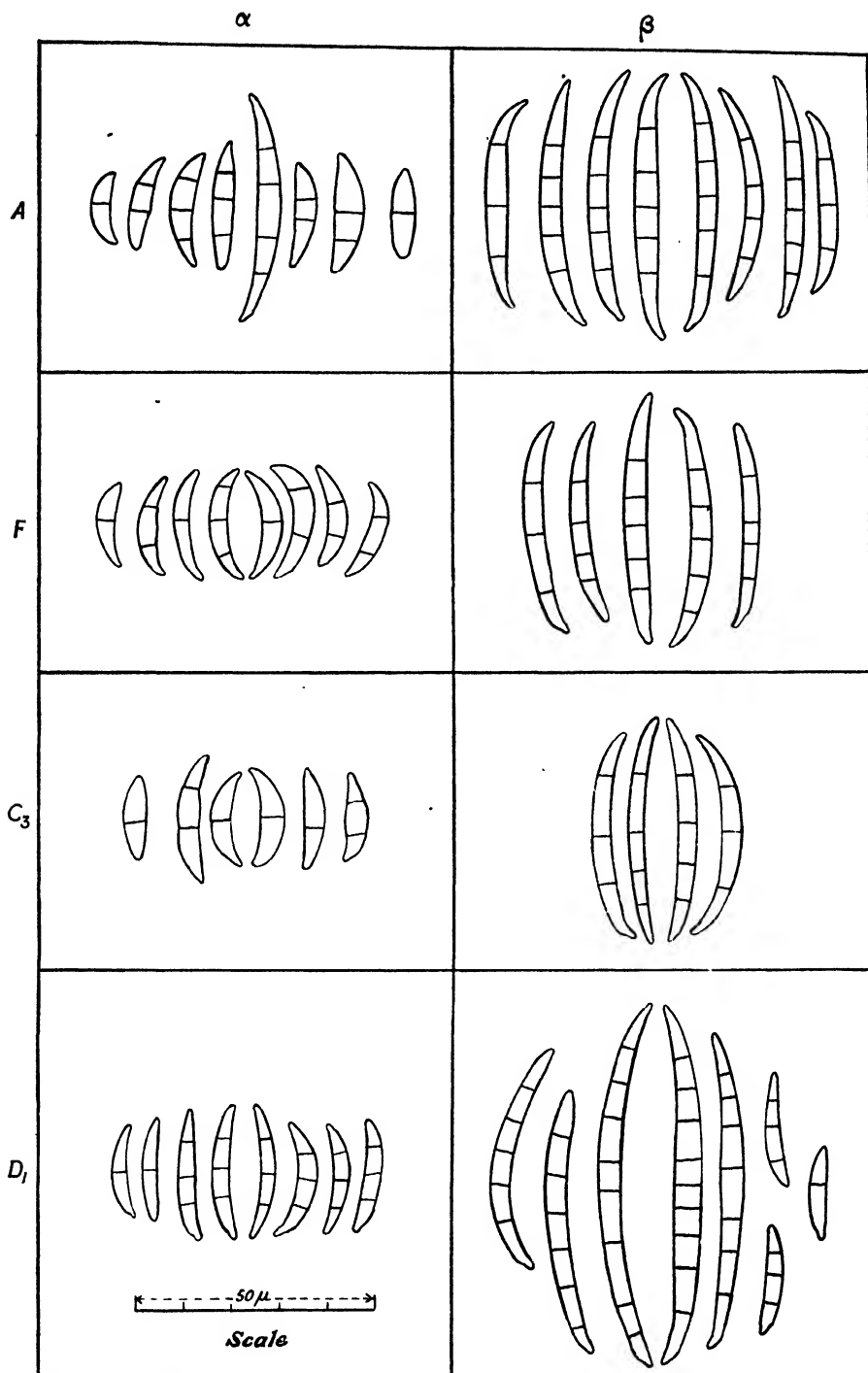
A certain group of these strains (the 'long-spore' type) behave in an unusual and characteristic manner when grown on a medium with a high C:N ratio. On the standard medium they produce a crop of fairly uniform spores with a high mode. When the C:N ratio of the medium is increased by diminishing the N content, the spores become increasingly heterogeneous, septations ranging from 0 to 8 or 9 being frequently met with in the same sample of spores. Table X gives the septation data for strain  $D_1$  grown on the synthetic medium in which the asparagin concentration is reduced.

TABLE X.

<i>Asparagin</i> <i>Concentration.</i>	<i>Septation.</i>									
	0-sep.	1-sep.	2-sep.	3-sep.	4-sep.	5-sep.	6-sep.	7-sep.	8-sep.	9-sep.
0.1 %	0	0	0	0	10	19	17	4	0	0
0.05 %	0	0	0	5	3	14	21	7	1	0
0.02 %	0	0	0	1	1	2	16	23	6	1
0.01 %	1	12	4	14	5	4	4	6	0	0

The heterogeneous nature of the spores on the media with high C:N ratio is well shown here. Though numerous spores of high septation are present under these circumstances, nevertheless the average degree of septation is comparatively low on account of the presence of large numbers of small low-septate spores. Figs. 17–21 of Pl. VIII illustrate the effect of changing the C:N ratio of the medium on spores of strain  $D_1$ . The effects shown are the same as in Figs. 12–16, which refer to strain A, with the difference that the spores in Fig. 21 are of the heterogeneous type above described. Fig. 11 illustrates still more strikingly the marked heterogeneity of the spores of this strain on a medium with a high C:N ratio.

The effect of the C:N ratio on the *appearance* of the spores, both as



TEXT-FIG. 3. Illustrating effect of asparagin content of medium on length and degree of septation of spores. Spores of strains A, F,  $C_3$ , and  $D_1$ , on media with high ( $\alpha$ ) and low ( $\beta$ ) concentration of asparagin.



regards their outline and the nature of their contents, is very striking. The following is a description of the appearance of the spores to which Table IX refers. The cultures concerned had been grown in the light at a temperature averaging  $17^{\circ}$  and were twenty-one days old at the time of observation of the spore characters.

*No asparagin added.* Spores highly granular, so that septa are invisible without staining; spores constricted at septa, and in many cases already germinated *in situ*.

*Asparagin 0.005 per cent.* Spores very similar to the last.

*Asparagin 0.01 per cent.* Spores still very granular, constrictions at septa less marked, and germination *in situ* very rare.

*Asparagin 0.02 per cent.* Spores less granular and no germination seen.

*Asparagin 0.05 per cent.* Granularity slight, so that the septa are clearly visible without staining; constrictions at septa no longer present.

*Asparagin 0.1 per cent.* Spores with hyaline contents.

*Asparagin 0.2 per cent.* Spores all showing moderate vacuolation; one or both of the end segments have an atrophied appearance.

*Asparagin 0.3 per cent.* Spores strongly vacuolated, and nearly every spore shows one or more atrophied segments.

*Asparagin 0.4 per cent.* Atrophy more pronounced still, involving in many cases the whole spore.

*Asparagin 0.5 per cent.* A very large percentage of the spores are completely atrophied.

The atrophied segments referred to in the above description have the following characteristics. With unstained spores the cell-wall shows up more faintly in the atrophied than in the normal segments. When the spores are stained with ruthenium red the protoplasm of atrophied segments is stained to a considerable extent, in contrast to the protoplasm of the normal segments, which remains almost unstained. The width of an atrophied segment is distinctly less than that of the others, presumably on account of loss of turgor. When the germination of spores containing atrophied segments is tested, the latter never put out a germ-tube. Spores in which all the segments show an atrophied appearance fail entirely to germinate and are obviously dead.<sup>1</sup> In many cases the atrophied segments disappear entirely; when the end segments, as is more generally the case, are so affected, the spores appear with truncated ends; when a middle segment is completely atrophied, the spore breaks up into two comma-shaped portions. Examples of spores with atrophied segments are to be seen in Pl. VIII, especially in Figs. 9, 18, 19, and 20.

The same series of changes in the appearance of the spores described

<sup>1</sup> Our thanks are due to Mr. J. C. Hopkins, B.Sc., for carrying out in this connexion a series of tests of the germinative capacity of spores from various types of media.

above is also obtainable when the asparagin content of the medium is kept constant and the concentration of the carbon constituent is varied. It is obvious, therefore, that the factor determining the nature of the spore contents and related appearances is not the absolute concentration of either the C or the N constituent, but the ratio of the two. Effects of the same kind, though to a less degree, are obtained when the source of nitrogen is potassium nitrate or ammonium chloride.

There is a definite correlation between the granularity of the spores and the intensity of colour formed in the medium. This is especially well shown when the C : N ratio of the medium is such that the central region of the colony remains colourless while a yellow zone is formed towards the outside. The spores formed in the colourless central region are distinctly less granular than those formed on the coloured region beyond.

In the case of those strains which produce a markedly heterogeneous type of spore on a medium with high C : N ratio, it is frequently found that the smaller spores have less granular contents than the larger ones. This is well shown in Fig. 21 of Pl. VIII. The same figure also illustrates the fact that the end segments of the spores tend to have less granular contents than the rest of the spore. Figs. 10 and 16 illustrate this effect to a less degree. This feature is undoubtedly related to the fact that the end segments of the spores are usually the first to show degenerative changes.

The tendency to vacuolation, followed by atrophy of particular segments and finally by complete death of the spore, is greatest when the C : N ratio is low. Such spores become vacuolate in a few days. With higher C : N ratio the spores are at first hyaline, and later become vacuolated, with subsequent atrophy. The granular type of spore obtained when the medium has a high C : N ratio has least tendency to degenerative change, so that such spores may remain unaltered for many months. Thus with increasing C : N ratio there is a continuous gradation in the direction of longevity of spores. Nevertheless, though many of the spores formed on a medium with low C : N ratio die comparatively soon, it is a remarkable fact that a small percentage may continue to show live segments for a long time. These segments are of the nature of chlamydospores, though there is no marked thickening of the wall observable, and certainly no double wall has been demonstrated for them in the case of any of the present group of *Fusarium* strains.

The presence of *constrictions* at the septa is seen when the C : N ratio of the medium is very high or very low; *vide* Figs. 11, 12, 17, and 21 of Pl. VIII. With media of an intermediate composition this feature is not shown.

At both extremes of the series, when the source of nitrogen is asparagin, there is a tendency for a certain percentage of the spores to germinate *in situ*; *vide* Figs. 11 and 17 of Pl. VIII. The latter tendency is probably due

to a slight acid reaction in the medium. In the case where the asparagin concentration is low, this acid reaction is produced by the metabolism of the fungus, and is permanent. In the case where the asparagin concentration is very high, the acid reaction is due to the acidic nature of the asparagin itself and only obtains for a limited time on account of the liberation of ammonia by the fungus. The association of the tendency to germinate *in situ* with constrictions at the septa is not surprising when one remembers that *Fusarium* spores in general show the constricted appearance when put to germinate in the ordinary way.

The correlation between *spore length* and degree of septation is obvious from a study of Text-figs. 2 and 3 and of Pl. VIII. Any change in growth conditions which increases the degree of septation also increases the average spore length, and vice versa. Table XI is a summary of data obtained from spores of strain A on various modifications of the synthetic medium.

TABLE XI.

<i>Septation.</i>	<i>No of Spores measured.</i>	<i>Average length (<math>\mu</math>).</i>
1	7	22.6
2	5	26.4
3	50	37.5
4	25	50.7
5	42	56.8

Exceptions to this rule are not uncommonly met with in the form of comparatively short spores with numerous closely set septa, some of the latter being frequently much finer than the others.

Little attention has been paid to the variation of *spore width*, but an examination of the figures in Pl. VIII will serve to show the kind of variation observed. Fig. 9 illustrates the swollen appearance frequently seen on strongly staling media. Figs. 11 and 21 illustrate the fact that in the case of the heterogeneous type of spore described on p. 216 the shorter spores are distinctly narrower than the longer ones.

The variation of *mycelial characters* has not been studied in any detail. The number of septa per unit length of mycelium is extremely variable in the same culture, so that no rule similar to the one governing the degree of spore septation has been made out. As regards the nature of the mycelial contents, there is a general correspondence between these and the contents of the spores, that is, the granular or vacuolated type of spore is associated with mycelium of similar appearance.

## SUMMARY.

1. A study has been made of the variability under different cultural conditions of certain microscopic features of *Fusarium* strains. In this con-

nexion special attention has been paid to the degree of septation of the spores.

2. There is a striking correlation between the intensity of staling shown by the fungus colony and the degree of septation of its spores. The staled type of growth is associated with spores of low septation, the unstaled with spores of high septation. This rule applies not only as regards different strains on the same medium but also as regards the same strain on different media. In accordance with this rule, low septation is produced by the following factors :

(a) High concentration of the nitrogenous constituent of the nutrient medium.

(b) Low concentration of the phosphate constituent.

(c) The presence of growth-retarding substances in the nutrient, such as an unduly high concentration of acid or alkali or of a toxic substance such as phenol.

(d) Increase of temperature.

3. The ratio of the concentration of the carbon to the nitrogen constituent is a factor of prime importance in determining not only the degree of septation of the spores but also the nature of their contents, whether vacuolated, hyaline, or granular. This factor also determines other characteristics, such as the presence or absence of constrictions at the septa, the tendency to atrophy and death, the tendency to germinate *in situ*, and the presence or absence of abnormal swollen segments.

4. A table is given showing the correlation between spore length and degree of septation.

5. A series of photographs and tracings of photographs is given illustrating the variation of spore length and of degree of septation under different cultural conditions.

## EXPLANATION OF PLATE VIII.

Illustrating Dr. Brown's and Dr. Horne's paper on *Fusarium*.

Figs. 1-4. Spores of strain A on media of concentration 4N, 3N, 2N, and N, respectively, where N is the standard synthetic medium. The composition of the latter is : glucose, 2 grm. ; asparagin, 2 grm. ; neutral potassium phosphate, 1.25 grm. ; magnesium sulphate, 0.75 grm. ; agar, 15 grm. ; water, 1 litre.

Figs. 5-7. Strains D, A, and D<sub>1</sub> respectively on the standard medium with 1 per cent. potato starch added.

Fig. 8. Strain D on standard medium with asparagin content reduced to 0.02 per cent.

Fig. 9. Strain C<sub>3</sub> on the standard medium four times concentrated.

Figs. 10, 11. Strain A and D<sub>1</sub> respectively on the standard medium with 1 per cent. potato starch added and concentration of asparagin reduced to 0.2 grm. per litre.

Figs. 12-16. Strain A on standard medium with asparagin concentrations 1 per cent., 0.5 per cent., 0.1 per cent., 0.05 per cent., and 0.02 per cent. respectively.

Figs. 17-21. Strain D<sub>1</sub> on the same series of media as in Figs. 12-16.



1.



2.



3.



4.



5.



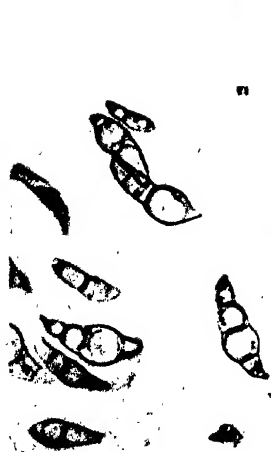
6.



7.



8.



9.



10.



11.

50 μ.



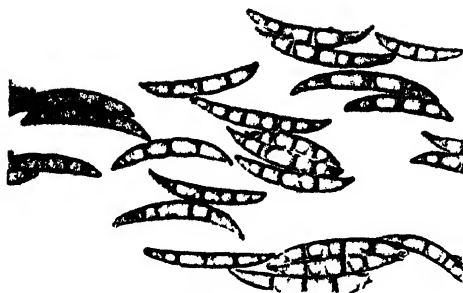
17.



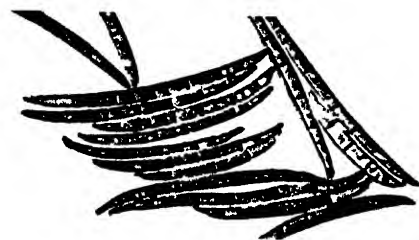
12.



18.



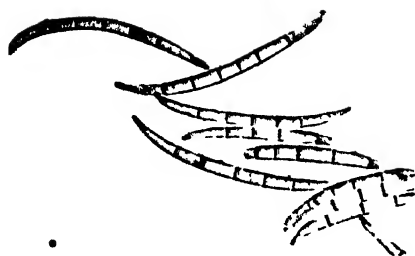
13



19



14



20



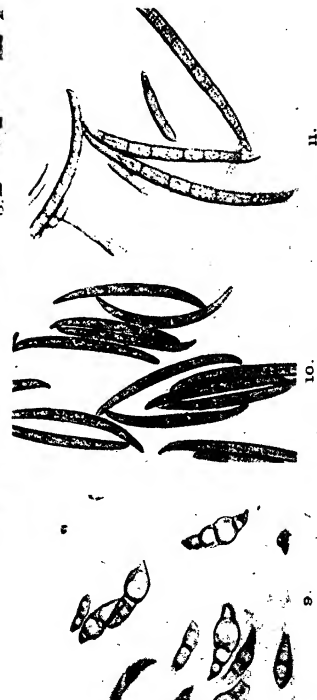
15



21.



16.



10. 50 μ.

BROWN & HORNE—FUSARIUM.



17.



husk cell.





# Studies in the Genus *Fusarium*.

## IV. On the Occurrence of Saltations.

BY

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With Plate IX and two Figures in the Text.

EARLIER papers in this series (7 and 8) have dealt with changes of cultural characteristics produced directly by environmental change. Such are usually described as modifications, and it is assumed that they are of a purely temporary nature, so that when the fungus is restored to its original environment its characters likewise revert to the original. Changes of a more lasting nature may be conceived as arising gradually as a response or adaptation to certain growth conditions, or by sudden jumps. The latter type of phenomenon, which is known to occur in a considerable number of fungal genera, and is usually described as a 'mutation', or more conservatively as a 'saltation', also occurs freely in certain circumstances in the present group of forms. These will be considered later in the present paper. Changes of the former type are more difficult to prove or disprove, as from the nature of the case they are small and perhaps only demonstrable after the cumulative effect of a series of reculturings. The effects, if they exist at all, are more likely to be of a quantitative than of a qualitative nature, and their demonstration will in general depend on a comparison of cultures of a certain date with those, it may be, of years before. Even with the most careful system of notetaking, such a comparison is not always easy, and it may not be possible to draw convincing conclusions. The best that one can do may be to record a general impression that the fungi have diverged in a certain direction from the form exhibited some time previously. It is obvious, of course, that comparisons at different times must be carried out under standardized conditions, and this, in the case at any rate of highly variable forms such as these *Fusarium* strains, necessitates the use of synthetic media. Ordinary decoctions such as potato agar are too variable in composition to be of any use for this purpose.

With a view to producing some definite evidence as to whether any change was taking place in the course of the routine culture of these *Fusarium* strains, a twofold series of tests was carried out. On the one hand, a careful determination of the growth curves of a number of strains under standardized conditions was made at a certain date, and after a somewhat lengthy interval of culturings the curves of growth were determined anew for the same strains under the same conditions, and a comparison instituted. In the other series of experiments a number of strains very similar to each other, but differing in certain particulars, were grown on a standard medium and a careful note made of the differences shown between them. At subsequent periods the same comparisons were made and evidence obtained as to whether they were changing their properties relatively to each other.

In these experiments, as well as in the whole of this work, the stock cultures were renewed at intervals of about six to eight weeks on a standard synthetic medium, and at each renewal spores were used whenever available. As all the strains used in the particular experiments under consideration sporulate more or less freely, the stocks were recultured from spores in every case. Throughout all this work the cultures of every strain have been carried on at one time or other from a single spore or its equivalent—more frequently from a single hyphal tip. As will be pointed out later, extreme precautions were taken to ensure freedom from admixture in these strains, and the majority of them have been repeatedly subjected to the process of renewal by carrying on from a single hyphal tip.

The standardized growth tests were carried out with four strains, A, F, C<sub>3</sub>, and D, which were chosen for this purpose because of the writer's familiarity with their general cultural features. As media the following strengths of the standard synthetic medium were used: 2N, 3N/2, N, N/2, N/3, N/4, N/5, where N represents the standard medium.<sup>1</sup> The less staling strains, A and F, were grown on the series of media 2N–N/3 and the more strongly staling strains, C<sub>3</sub> and D, on the series of media N–N/5. The cultures were incubated at 20° and the diameters of the colonies measured at about two-day intervals. Fig. I gives the curves obtained at the first determination, which took place in October 1922, and Fig. II those obtained when the test was repeated during March/April 1924. In both figures the various concentrations of the medium are spaced at equal distances along the *x*-axis, while the ordinates represent the diameters of the colonies at the various intervals.

A comparison of Figs. I and II brings out very strikingly the similarity in behaviour of the various strains after an interval of nearly a year and a half. Strain A in both cases shows the least degree of

<sup>1</sup> Composition: glucose, 2 grm.; asparagin, 2 grm.; neutral potassium phosphate, 1.25 grm.; magnesium sulphate, 0.75 grm.; agar, 15 grm.; water, 1 litre.

staling, and the other strains follow in the order F, C<sub>3</sub>, and D. The difference in staling capacity between strains C<sub>3</sub> and D is slight, and is only shown by the fact that the latter stales on the medium N/2, whereas the former does not. This feature is shown strikingly in both figures. The only difference between the growth curves in the two figures consists in the slightly slower growth of all the strains in the case of the later determination. A slight variation in the temperature of the incubator is probably responsible for this, as it is known from other determinations that there is no evidence that the intrinsic rate of growth of these strains is tending to fall off.

It was pointed out in the earlier papers of this series that the degree of staling shown is correlated with certain other cultural features, e. g. the amount of mycelium, amount and distribution of sporing, degree of septation of the spores, &c. The fact that the staling features of these strains have not changed is presumptive evidence that those characters which are correlated with staling have also remained unaltered. When to this one adds the general impression that these strains behave now as formerly, there are considerable grounds for the statement that they have not changed in any material respect in consequence of the routine cultural methods adopted.

In the second series of experiments a number of closely similar strains derived as saltants from the original strain A were used. Comparisons were made of the cultural appearances of these strains on two media, (1) the standard synthetic medium, (2) the same modified by the addition of 1 per cent. potato starch and by reduction of the asparagin content to 0.02 per cent. The object of the latter medium was to test the colour reactions of the strains. The eight strains tested differ from each other chiefly in the amount of sporulation and of aerial mycelium formation on the standard medium and in the amount of yellow colour production on the medium modified as described above. The kind of differences shown will appear more clearly from the following table:

TABLE.

<i>Strain.</i>	<i>Appearance on Standard Medium.</i>	<i>Colour.</i>
A <sub>7</sub>	Occasional sporodochia round centre of colony; otherwise entirely sterile mycelium.	Feeble yellow.
A <sub>3</sub>	Large mass of spores at centre of colony; mycelium beyond	Moderate yellow.
A <sub>2</sub>	Similar to last	Strong yellow.
A <sub>4</sub> )	Sporulating region more spread than in preceding strains, but distinct zone of aerial mycelium round margin	Good yellow.
A <sub>5</sub> )		
A	Sporulation over whole colony except for faint ring of aerial mycelium round margin	Good yellow.
A <sub>6</sub>	Intense sporulation over whole surface; no mycelial fringe	Feeble yellow.
A <sub>1</sub>	Moderate sporulation over whole surface	No yellow.

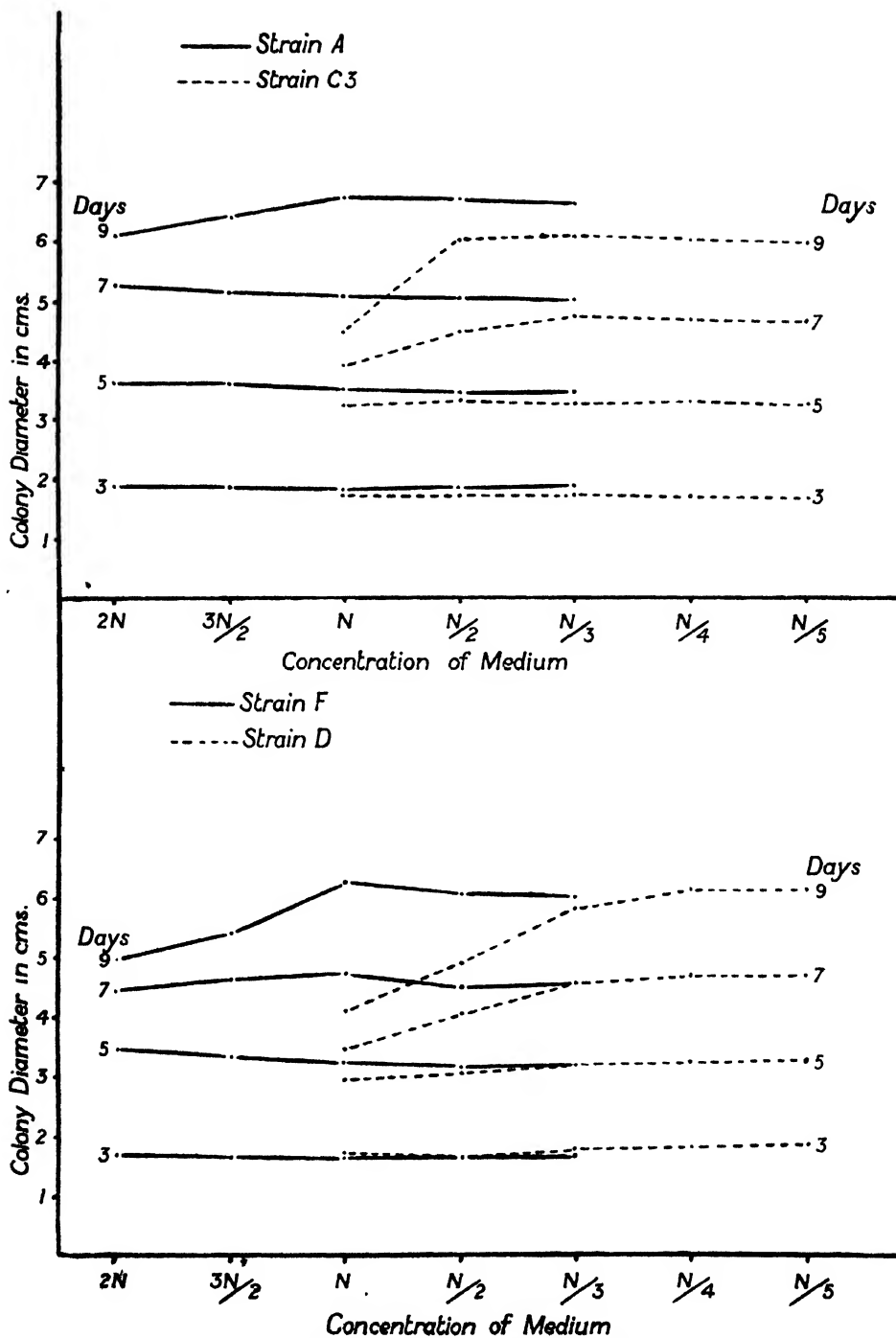


FIG. I. Growth curves of strains A, F, C<sub>3</sub>, and D on various concentrations of the standard medium as determined during October 1922.

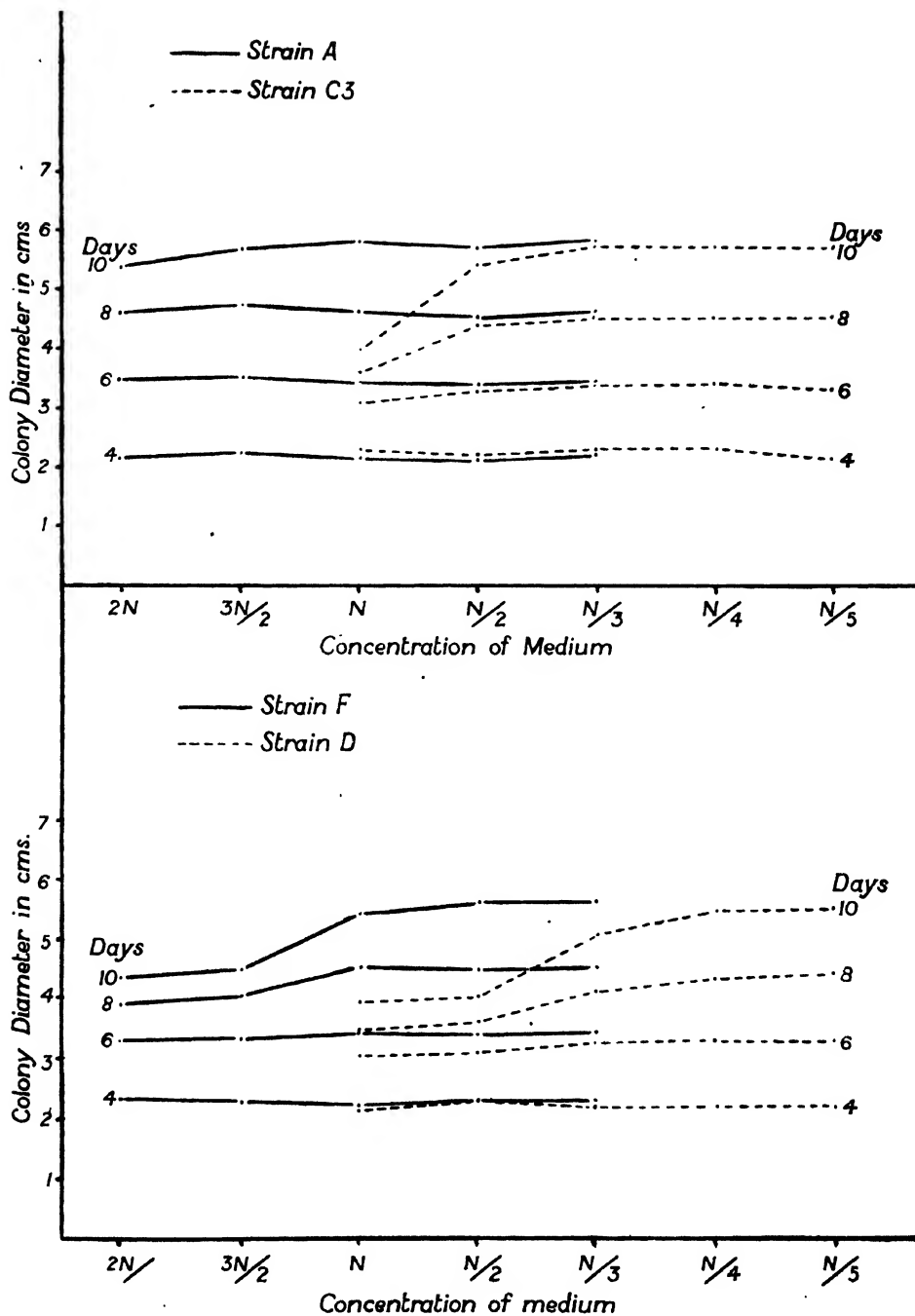


FIG. II. The same series of curves as in Fig. I, as determined during March/April 1924.

The stock cultures of these strains were kept as usual in tubes of the standard synthetic medium, and tests were made in plate cultures at the following times: in December 1923, February 1924, June 1924, and February 1925. The description given in the table was found to be true at each examination, with the single exception of strain  $A_3$  at the last date mentioned. Here the well-known radial or sectored appearance was shown, indicating that saltation had taken place at some period of the reculturing process, so that the culture was now mixed. Strains  $A_4$  and  $A_5$  from the beginning were so similar as only to be doubtfully different, and one of them might well have been discarded. However, it was decided to keep both for purposes of the present test in order to see if they diverged in any way. No such effect has taken place. The interest of the present experiment lies in the proof afforded of the fact that strains, some of which only show slight differences from each other,<sup>1</sup> can be maintained as such if suitable cultural methods are adopted.

As has already been stated, spore inocula were always used, when available, for the process of routine reculture. However, some of the strains sporulate so feebly that mycelial inocula, composed either of aerial mycelium or of mycelium with a portion of the substratum, had in some cases to be used. It was decided to carry through a series of experiments to determine whether any variation of a more or less permanent nature could be produced by the selective use of different kinds of inocula. Special attention was directed to a comparison of cultures derived from spore inocula with those from mycelial inocula, with a view to determining whether in the latter case the strain tended to lose its sporulating capacity: an effect which appears to take place in the case of some fungi, especially of those which have pycnidial or perithecial fructifications.

The following account of a particular experiment will illustrate the method. Strains  $A$  and  $C_3$ , when grown on the standard synthetic medium, sporulate strongly, especially when cultured in the light. When the glucose concentration of the medium is raised to 8 per cent., and when the cultures are grown in the dark, the former strain produces sporeless colonies, while those of the latter are nearly so. A parallel series of reculturings was therefore carried out with both strains, in the one case on the standard medium in the light via spores at each transfer, and in the other on the medium reinforced with glucose in the dark via mycelium at each transfer. The strains were in this way carried down through six reculturings at fortnightly intervals. At the end of the period transfers of spores in the one case and of mycelium in the other were made to the standard medium

<sup>1</sup> It may be pointed out that the strains described in the table on p. 225 constitute a series, ranging from an almost completely mycelial form ( $A_7$ ) at one end to forms devoid of aerial mycelium and more or less covered with a layer of spores at the other end. The colour effects are also arranged in series, being slight at both ends of the series and strong in the middle.

and all the colonies grown side\*by side in the light. The results were slightly different in the case of the two strains.

*Strain A.* There was no difference whatever between the cultures which had been treated as described above.

*Strain C<sub>3</sub>.* A certain amount of difference did appear. The colonies derived from the mycelial transfers showed a slight but at the same time consistent reduction of staling capacity as compared with those which had been recultured through spores. Again, the former lagged behind the latter in the replacement of aerial mycelium by spores, so that for a time the colonies derived from mycelium showed a less sporulating capacity. After a time, however, this difference practically disappeared, and when in the next generation spores were taken from the two series the colonies produced were identical. Further experiments showed that the same kind of difference could be obtained between spore and mycelial inocula without the process of selective reculturing described above. This strain in fact, as contrasted with strain A, illustrates the case where there is a slight intrinsic difference between spore and mycelial inocula, the colonies produced not being quite identical in the two cases. The difference, however, is small, and there is no evidence that the process of repeated selective culturing intensifies it. Also the difference so produced disappears at once when similar inocula are taken.

A large number of tests has been made from time to time on the question of a possible difference between spore and mycelial inocula with a number of other strains, especially with a strain D, which, as will be shown later, can be made to saltate freely and produce widely different permanent strains. When grown on the standard medium this strain typically shows a pustule of spores in the centre of the colony, then a zone devoid of spores, and beyond that a strong ring of spores. The central pustule of spores is variable in size and is frequently absent. Also in some cases a certain amount of diffuse sporulation occurs over the general surface of the colony. Attempts to correlate these variations with the nature of the inoculum, whether of spores or of mycelium, failed; mycelial inocula were found to give the more strongly sporing colonies as often as inocula consisting entirely of spores. Thus in this strain, though there is a certain amount of unexplained variation, there is no definite difference between the potentialities of spore and mycelial inocula.

All the strains that have been tested behave similarly to one or other of the ones described. Thus it appears that any intrinsic difference between spores and mycelium, from the point of view of the colonies to which they give rise, is non-existent, or at the best of negligible amount.

In the present connexion, comparisons were also made between spores derived from media of widely different composition. The tests were

carried out with modifications of the standard synthetic medium. The following illustration will suffice: Strain A was grown on two media, one with very low and the other with very high asparagin content. The colonies produced on the medium with low asparagin content developed after some days a strong yellow colour throughout the medium, while those on the medium with high asparagin remained colourless. When the colonies were two months old, spores were transferred from each to a medium with low asparagin content, in order to determine whether any loss of colour-producing capacity had taken place as a result of culturing on the medium with high asparagin content. The spores derived from the latter medium were slower in germinating than those from the other, an effect which can be described as attenuation due to the liberation of ammonia in the cultures with the high asparagin content. The resultant colonies were therefore, throughout their growth, somewhat behind the others in area, and the appearance of the yellow colour was delayed by about two days. The final result was, however, identical in the two cases.

From these results, therefore, it appears that in the case of these *Fusarium* strains the physiological state of the inoculum has little or no effect on the appearance of the resulting fungal colony, and that new strains are not likely to be produced by selective culturing of the type above considered.

While there is little evidence of any slow progressive changes arising in the course of the culture of these organisms, changes of the sudden type known as 'mutations' or 'saltations' have been met with a considerable number of times. The remainder of this paper will be taken up with a consideration of these results.

That saltations do occur in monospore cultures of fungi has been definitely established by a number of investigators. Thus Crabill (11), working with *Coniothyrium pirinum*, found that a certain strain was liable from time to time to throw a new one, which in subsequent culture remained always different from the parent, and showed no signs at any time of reversion to the original. This saltation was accompanied by a very considerable change in the morphology of the fungus, e.g. the parent form possessed numerous unilocular pycnidia, while the saltant had few multilocular pycnidia.

Stevens and Hall (18) record a radial appearance in cultures of *Ascochyta chrysanthemi*. This appearance suggests the occurrence of saltation, but apparently no means were taken to ensure the isolation of the two strains, and subsequent recultures behaved in an unintelligible manner.

Chaudhuri (10) describes a saltation in a species of *Colletotrichum* which differed from the parent in its colour reactions, and in the size, distribution, and abundance of sclerotia produced. The saltant maintained its properties on certain media, but in some cases, as on potato mush agar, it reverted to the original form.



Burger (9) in a study of some naturally occurring forms of *Colletotrichum gloeosporioides* found that several of these saltated in course of culture, and gave in some cases forms indistinguishable from naturally occurring varieties. The sectored appearances in culture, as figured by Burger, show a strong resemblance to what has been met with in the present study of *Fusarium*.

Brierley (2) records the sudden appearance in a culture of *Botrytis cinerea* of a strain with white instead of the normal black sclerotia. This new strain kept constant in culture.

The fullest account of the occurrence of this saltation phenomenon is given by Stevens (17) in a paper dealing with the culture of *Helminthosporium sativum*. Practically any cultural feature was found to undergo sudden change in this way. Some of the saltants proved to be very stable in culture, while others were very variable. Occasionally a saltant reverted to the original. No evidence was found of a change of parasitic power arising by saltation, but this question was not examined critically. Stevens's results are very similar to those met with in the present study.

Appel and Wollenweber (1), in an important memoir on the classification of *Fusarium* species, describe certain results which, as will be pointed out later, indicate that saltation was taking place, though the authors did not apparently recognize it as such.

A study of fungal literature reveals numerous instances where saltation probably has taken place, but the above citations will suffice. The phenomenon of saltation is not confined to fungi, but occurs freely among bacteria. The literature on this subject is summarized by Dobell (12).

Throughout the whole group of the fungi it is becoming increasingly known that a large number of species contain within their limits a considerable variety of strains. Good illustrations are to be found among species of *Aspergillus* (Thom and Church, 19), *Glomercella* (Edgerton, 13; Shear and Wood, 15), *Rhizoctonia* (Matsumoto, 14), *Cladosporium* (Brooks and Hansford, 5), *Puccinia* (Stakman and co-workers, 16), &c. It is highly probable that in many cases some of these strains can be derived from others in artificial culture.

During the course of this work, while the occurrence of saltations was being specially attended to, extreme precautions were taken to ensure the freedom of all the strains from admixture. At each renewal the stock cultures were transferred, first of all, to plates of plain agar, and from these hyphal tips were cut out by the technique described in an earlier paper by the writer (6). These hyphal tips formed the starting-point of the next series of stock cultures. This process was carried out at six-weekly intervals or thereabouts, over a period of fully one year, so that the strongest evidence was forthcoming that all the strains were each single mycelia. Whenever a saltant strain appeared, both the saltant and the parent strain

were picked up and carried on, each from a single hyphal tip. This precaution, though necessary for guaranteeing freedom of each strain from admixture, is in many cases probably quite superfluous, since the ordinary process of transfer, as a rule, gives apparently pure colonies.

It may be mentioned here that the group of *Fusariums* under consideration have one special feature which particularly recommends them for the study of saltation. That is, that they have no air-borne type of spore. The conidia are borne in wet masses, and do not blow about. Thus it is that not once in the course of thousands of culturings in Petri dishes has one of the strains appeared as an accidental contaminant in any of the dishes. The case of *Penicillium* offers a pointed contrast in this respect. There is little doubt that saltations do occur in *Penicillium*, but very special precautions would be required to guarantee that a radial or sectorial appearance in a colony represented a genuine saltation, and was not due to the accidental falling in of a strange spore which happened to alight at a suitable spot in the neighbourhood of the growing edge of the colony.

Saltations have occurred from time to time in an apparently accidental manner in the course of this investigation. Relatively to the number of cultures made, the number in which saltation appeared was insignificant. Later it was found that, by using a particular type of nutrient medium, saltations could be produced at will, especially in the case of certain strains as parents. Before dealing with these latter results, the general appearance of saltations, as they show themselves first in culture, will be illustrated by a discussion of the photographs reproduced in Plate IX.

Fig. 1 illustrates the simplest type of saltation. The culture figured was on a medium favourable for colour production. The parent strain, which in the present case occupies the bulk of the culture, is characterized by fairly strong sporulation in sporodochia, and by intense formation of yellow colour. The saltant, which appears as a small diverging sector, with apex at some distance from the centre of the colony, is characterized by less staling capacity, by absence of the yellow colour, and by diffuse, somewhat feeble sporulation. The tendency of the saltant sector to increase at the expense of the parent is probably due to its smaller staling capacity. Whether the actual saltation took place at the apex of the sector, or whether the saltant was already present in the inoculum and only asserted itself at some distance from the centre, is a matter which cannot readily be determined. As will be pointed out below, appearances very similar to the present are shown in cases where the mixing of strains must have already existed in the inoculum, though the separation of the colony into distinct sectors did not take place until some distance from the centre. On the other hand, sectors have, in a few cases, been obtained in cultures of which the inoculum was a single hyphal

tip, in which case it appears highly probable that the actual saltation took place at, or near the apex of the sector. In the case illustrated in Fig. 1 the probabilities are that the saltation took place during the growth of that particular colony, as this was the only one out of a batch of four which showed the effect.

Fig. 2 shows an appearance very similar to Fig. 1, but with the important difference that the sector which occupies only a small portion of the colony is the parent form. In the duplicate plate the entire colony consisted of saltant. The colony figured here, as well as those of Figs. 3-7, developed from inocula taken from cultures on Richards's solution agar, which were each planted with single hyphal tips.

The strain which occupies the great part of the colony is an intensely sporing one, whereas the parent is of the mycelial type. It is obvious that in the present case a process of haphazard reculture would lead to the elimination of the parent form altogether. This statement applies *a fortiori* if, as is often the case, one uses spores wherever available for reculturing purposes.

Infection experiments which are at present in progress in this laboratory show a very pronounced change in parasitic power in some of these saltants as compared with the parent, so that there is here a strong indication of the manner in which fungal cultures change their infective power. This point will be discussed more fully later.

Fig. 3 illustrates the same relations as Fig. 2, except that the parent appears as three distinct sectors. Careful study of the photograph shows one, if not two, places where the parent strain is present, though it has failed to assert itself.

Fig. 4 is similar to Fig. 1, except that the sector begins from the centre. The parent is a mycelial staling form. The saltant is a form which sporulates freely in sporodochia, and shows less staling reactions. In consequence of this it is tending to spread at the expense of the parent.

Figs. 5 and 6 are similar to each other, and illustrate the case of an intensely sporing and strongly staling form appearing as saltant in a mycelial strain. The latter in both cases is apparently entirely submerged at the centres of the colonies, but in virtue of its less strongly marked staling reactions it is tending to cut off the saltant. Various stages in this process are shown in these figures. The two strains present in Figs. 5 and 6 are the same as in Figs. 2 and 3, and the different appearances are no doubt associated with the relative amounts of each strain present in the inocula, the parent form being presumably more represented in the case of Figs. 5 and 6.

Fig. 7 is a more complicated case where there are at least three strains present. The parent mycelial strain occupies most of the right-hand side of the colony as figured, and also occurs as small areas between the saltant

sectors. The latter consist of an intensely sporing pionnotal form (pointing towards the upper left hand) and three sectors of a sporodochial strain (the sporodochia shown as dark spots on the photograph) which are spreading at the expense of the rest of the colony.

Fig. 8 is similar to Figs. 5 and 6. The medium in this case is one suitable for colour production. The three radiating clear areas represent the saltant, which is characterized by pink colour and diffuse sporulation. The remainder, which is the parent, is almost devoid of spores and has a strong yellow colour.

Figs. 9, 10, and 11 illustrate complicated cases of saltation, in which the individual strains are not always clearly separated from each other.

In Fig. 9 the colony covers the whole plate, and consists mainly of a form which sporulates thinly and diffusely. All this is saltant. Half encircling the centre of the colony is a ring of sporodochia, and at a few places over the colony are isolated sporodochia. These belong to the parent form, and by careful transfer the latter was easily recovered from these.

Fig. 10 is similar to the preceding. The groundwork of the colony is again the saltant, and the parent can be recovered from the scattered sporodochia or from the tufts of aerial mycelium showing here and there on the surface.

Fig. 11 is a complicated mixture. The colony covers the whole plate. A diffusely sporing form covers the central region, and runs out in a broad band to the margin. This is a saltant. Elsewhere the outer part of the colony is occupied by a faintly coloured form with occasional small sporodochia. This also is a saltant. Along the apparent junction of these two strains are a number of clustered sporodochia. These give the parent form.

The diffusely sporing form which covers nearly the whole surface in the case of Figs. 9 and 10 and a considerable part of the surface in the case of Fig. 11 has, in contrast to the parent form, extremely weak parasitic powers on the apple fruit. It is obvious that haphazard reculture from these plates would certainly lead to elimination of the parasitic form, and to the carrying over of a purely saprophytic strain. It is suggested that some such effect as this takes place where, as often happens, it is reported that a parasitic fungus loses its virulence in culture. If what has just been described in the case of *Fusarium* is at all general, the true facts are, not that the fungus has lost its virulence in culture, but that a saprophytic saltant has been substituted for the virulent strain. If sufficient attention is given to the stock cultures, and especially if the appropriate type of medium is used, there does not seem to be any reason why parasitic strains should 'go off' in artificial culture. Suggestions will be put forward later in this paper as to the best means of ensuring stability in fungal cultures.

It has been already mentioned that some saltants arose from time to time on a variety of media in the course of general cultural work. Later it was found that on certain media saltation took place very frequently in the case of certain strains. The medium which proved to be most suitable for producing saltations was Richards's solution agar.<sup>1</sup> A considerable number of experiments were carried out with this medium along the following lines:

A strain D was chiefly used in this connexion. A single hyphal tip was placed in the centre of a large Petri dish containing Richards's solution agar, and the colony allowed to grow at laboratory temperature in the light. To keep the colony free from air-borne contamination, the Petri dish was enclosed in a larger Petri dish. The developing colony was then sampled from time to time, i.e. pieces of aerial mycelium, or of spores, or of sclerotia, &c., were transferred to plates of the standard synthetic medium and the identity of the strain determined from its appearance on the latter.

On Richards's solution agar, strain D forms a colony which covers the whole plate. As time goes on the mycelial web thickens up to form a strong felty mass (plectenchyma), which becomes wrinkled and cracked over the central region especially. Here and there, in a quite irregular manner, irregular sclerotium-like bodies appear which become blue. Sporulation is in the form of sporodochia scattered sparsely and irregularly over the whole surface. In addition, spores appear in thin bands along the edges of the cracks of the mycelial web; there is invariably a zone of spores on the glass round the margin of the colony; spores appear on the top of some of the sclerotia, and occasionally a broad effused area of spores may be shown. The centre of the colony is typically occupied by a large pustule of spores. The method of experiment consisted in taking inocula from different marked parts of the colony, planting these on plates of the standard synthetic medium, and observing what kind of colony developed. Later in the experiment the same regions of the colony were again tested, and the results compared with the earlier ones. Though there were no obvious saltations in the form of sectors in the parent colony, nevertheless it was found that inocula, whether of spores or of mycelium, taken from different marked spots of the parent consistently behaved differently from each other. Thus spores taken from one particular spot would consistently give a saltant usually mixed with the parent, while an apparently similar mass of spores elsewhere gave the parent only. As the parent culture increased in age, the percentage of spots which gave indications of saltation tended to increase in number. The general results are best illustrated by a particular example.

<sup>1</sup> Composition: cane sugar, 50 grm.; KNO<sub>3</sub>, 10 grm.; KH<sub>2</sub>PO<sub>4</sub>, 5 grm.; MgSO<sub>4</sub>, 7 H<sub>2</sub>O 2.5 grm.; iron salt, a trace; agar, 15 grm.; water, 1 litre.

The parent colony was derived from a hyphal tip of strain D. Medium, Richards's solution agar.

*First sampling, after 11 days.* Inocula were taken in the form of spores from the centre of the colony, of aerial mycelium from half-way to the growing edge, and of growing tips from the edge of the colony. All produced the parent strain only.

*Second sampling, after 5 weeks.* Again no trace of saltation was found.

*Third sampling, after 9 weeks.* Out of fourteen spots tested, the parent strain came up pure in ten cases, in the remaining four a saltant was present.

*Fourth sampling, after 13 weeks.* Twenty-one inocula were taken. Thirteen of these gave the parent, and eight gave saltant forms.

*Fifth sampling, after 17 weeks.* Of eighteen inocula taken, eight gave the parent and ten gave saltants.

These saltants were of course not all different from each other. In fact the same saltants appear consistently in the same place. Altogether in this experiment ten of the saltants were carried on as possibly being different from each other. These were purified by the hyphal tip method and then grown on a number of test media for purposes of comparison. Five of them proved to be definitely different from each other. A further test showed that two of these were identical with strains already got from the same parent, so that altogether the yield from this experiment was three new strains.

This type of experiment had been carried out with strain D five times, with a similar result on each occasion. Other strains gave similar results, e. g. strains A, B, and E<sub>1</sub>. Some strains give only one saltant, and the strain C<sub>3</sub> gave none at all. This last strain is peculiar in that it frequently shows vague indications of the sectored appearance, especially on colour-producing media. Numerous attempts have been made to isolate a saltant from these sectors, but the result has been simply the parent in every case. This strain appears to be one of the most stable in the group.

In the course of these tests a careful record was kept of the position on the parent culture from which the various inocula were taken, and a note was made of the nature of the inocula, whether composed of spores only, or of mycelium only, or of a mixture of the two. But only a limited amount of rule was made out in this connexion. As was to be expected, the intensely sporing form of saltant arises from spore inocula, and the sterile type of saltant from mycelial inocula. Thus, if saltation has taken place in the parent culture, selection of spore inocula will tend, if anything, to intensify sporulation, whereas by choosing aerial mycelium there is a risk that a sterile strain may be picked up. But except in such a suitable

object for showing saltation as strain D, and then only in old cultures, the majority of inocula, whether consisting of spores or of mycelium, give the parent only. In other words, the differentiation as between spores and mycelium considered as inocula has no necessary connexion with the development of new strains in culture. Of two apparently similar masses of spores, one would give the parent and the other a saltant. An attempt was made to relate the saltation effect with the nature of the underlying growth, whether loose mycelium, or plectenchyma, or sclerotium, but no rule was manifested.

The general conclusion is that on such a medium as Richards's solution some of these strains, even when put on in the form of a single hyphal tip, develop into a heterogeneous colony, though there may be none of the sectoried appearance showing. While the greater part consists of the parent type, small areas exist scattered over the surface, where apparently some kind of segregation has taken place, and from which one can readily pick up new strains. The latter are less likely to be found in young cultures, and, it may further be added, are more frequently met with in the central region of the culture than towards the periphery.

In the light of the above results it seems hardly necessary to emphasize that, for fungi such as the *Fusarium* strains dealt with here, a medium like Richards's solution agar is highly unsuitable if the aim is to preserve the fungi in their original form. In the case of strain D grown on this medium it was found that in old cultures it was about an even chance whether a particular inoculum taken from it gave the parent in the pure form or the parent mixed with some saltants. It is obvious that in such circumstances the parent form will inevitably be lost in the course of a few subculturings, and replaced by a saltant or a mixture of them. This will be accompanied in some cases by a very distinct change in the physiological and pathological reactions of the organism.

For the purpose of producing saltants, Richards's solution agar has proved to be the most serviceable, that is, the actual number of saltants that were isolated from cultures on this medium proved to be greater than from, for example, potato mush agar, though this result may not have general application. On the other hand, ordinary potato extract agar (200 grm. potato to the litre) and still less the synthetic potato extract used by the writer are not suitable for producing saltants. In other words, the latter media are more suitable as stock media for preserving these strains. Saltations do occur on these media, but comparatively rarely. The following illustration is sufficient to emphasize this point: The strain A has been kept in culture since 1919, for part of the time on potato extract agar and later on the synthetic medium. During that time many thousand plates of this strain have been examined in the course of physiological studies and only on three occasions has it shown the sectoried appearance indicating

saltation. Two of these saltants proved to be the same, so that altogether two new strains were obtained. On the other hand, a single culturing on Richards's solution agar in which the inoculum was a hyphal tip of strain A gave on analysis at least four new strains, all of which have since maintained their characteristic features when cultured on the synthetic modification of potato extract agar.

As compared with the parent the various saltants may show intensification or reduction in respect of any particular feature, that is, saltation may take place in either direction. Thus the strain A, which on a certain medium gives moderate colour development, has as saltants the strain  $A_1$ , which forms no colour, and  $A_2$ , which forms intense colour. Strain A on a certain medium forms little aerial mycelium, a saltant  $A_6$  forms none, another saltant  $A_7$  forms a considerable amount, and similarly in converse ratio with regard to intensity of sporulation. Strain A has weak staling reactions, the saltant  $A_1$  has still less, whereas  $A_7$  stales strongly. Similarly the strain D, which sporulates moderately, has saltated into intensely sporing pionnotal forms and into strains which are almost sterile. Nevertheless, no case has been seen where, say, a strain I saltated to a strain II and the latter subsequently saltated back to I. Stevens, in the paper cited, records such cases, and the fact that fungi which have lost their parasitic power in culture can be restored by appropriate treatment to their original vigour is probably to be interpreted in this manner. In the present work, such reversions have not been sufficiently searched for, as most of the culturing on the Richards's solution medium was done with the original strains themselves. Saltant strains have from time to time given other saltants, but these have always proved to be different from the original parent.

The following case is worthy of mention: A strain which may be indicated as I saltated on two occasions, giving two different strains, II and III. The strain II itself subsequently saltated, giving a strain which was not definitely distinguishable from the strain III. Thus the original strain reached a certain result in one case by one jump, and in the other by an intermediate stage.

An interesting physiological feature often seen when saltation is accompanied by the sectored appearance is that the cultural features of the strains are intensified at their junction. Fig. 2 of Pl. IX is an illustration of this effect. The bulk of the colony consists of a strain which forms a more or less continuous layer of spores over the surface; the sector consists chiefly of aerial mycelium. Along the line where the strains meet there is a pronounced ridge of spores. Similarly, when the sectored appearance occurs on a medium which gives rise to colour formation, the intensity of the latter tends to be greatest in the neighbourhood of the junction of the strains;



GENERAL DISCUSSION.

A certain amount of discussion has taken place recently as to the nature, from the genetic point of view, of the phenomenon of saltation. In a number of recent addresses Brierley (3, 4) has dealt with this question in considerable detail. Brierley takes exception to the use of the term 'mutation' for this phenomenon, inasmuch as the criteria of purity which are usual in the study of the genetics of higher plants cannot as yet be applied to the case of fungi or bacteria. He considers that cases of so-called 'mutation' are simply segregations from a heterozygous parent. By a series of arguments based entirely upon analogy and with no definite experimental evidence pointing one way or the other, Brierley is led to suspect the presence of the heterozygous condition as more or less universal throughout the fungi. The chief argument for this contention is the known occurrence of sexual fusions in certain fungal groups, and of hyphal fusions in the mycelia of fungi generally. The latter fusions, according to this theory, are visualized as leading to a heterozygous condition in part of the mycelium, though in the present condition of mycological technique it has not been found possible to bring forward any evidence bearing on this point. The fact that these saltations occur freely among the bacteria in which no process of fusion of any kind has ever been observed offers considerable difficulties to the theory of 'heterozygousness', difficulties which Brierley meets with a series of speculations still less capable of proof or of disproof than in the case of the fungi. Such being the case, it is not clear what useful purpose is served by speculative discussion of this type, which has no conceivable practical bearing on the study of mycological problems either at the present day or for many years to come. The genetic nature of the saltation effect being wholly unknown and perhaps unknowable, it is perhaps as well that the non-committal word 'saltation' should have been coined (apparently by Stevens) for their designation, but the present writer is far from sharing in Brierley's alarm at the lamentable consequences which the unblushing use of the word 'mutation' would entail to mycological science. It is obvious that this word has been used by mycologists and bacteriologists to crystallize what is the salient feature of these changes occurring in culture, viz. that they are sudden changes which are neither the result of a process of gradual acclimatization or 'education', nor of a mere sorting out of one strain from what was originally a mechanical mixture. Used in this sense, and this is the only sense in which the term 'mutation' can be applied as yet in microbiology, no misconception need be feared. The relation of such 'mutations' to hypothetical changes in the chromatic material of nuclei, which latter in the case of bacteria have themselves little more than hypothetical existence, is a matter which does not seriously interest the practical student of fungi or bacteria.

To most workers the interest of these phenomena lies in other directions. Saltations have a threefold interest. In the first place they directly concern the systematist. It is only by knowing the limits of variation of a form and its saltants that the limits of the species can be mapped out. This has not been recognized in much descriptive work, and in consequence many forms have been described as species which more detailed work would in all probability show to be derivable from other forms by saltation. A way would thus be opened for a much-to-be-hoped-for reduction in the number of fungal species which are now listed and the number of which is constantly being added to. In the second place, the phenomenon of saltation is of great interest to the pathologist, inasmuch as saltant forms may differ markedly from the parent in respect of their virulence. The bearing of saltation upon epidemic outbreak of disease is thus obvious. Lastly, the question of the possibility of saltation in cultures of micro-organisms is one of very great practical importance to the biologist whose concern it is to study disease phenomena by the laboratory method.

A few suggestions on the last-mentioned question, based upon the experience gained in the course of the present research, may fittingly conclude this discussion. The important point has been brought out in this work that the tendency of these *Fusarium* strains to saltate is a function of the cultural medium. On a weak glucose-asparagin-mineral salts medium saltation takes place quite rarely, so that with a moderate amount of attention the individuality of all the strains can be preserved on this medium. On the other hand, a concentrated synthetic nutrient (Richards's solution) was found to predispose strongly to saltation, so much so that it would be impossible, without extraordinary care, to preserve some strains on this medium for more than one or two generations. The latter medium is thus the one to use for the production of saltants, the former is the one on which to preserve them when they have been produced. The different behaviour of the two media is perhaps not entirely due to different concentration. The following is a suggested interpretation. It has been noted above that the proportion of inocula which give rise to saltants increases with the age of the culture and is greater in the central region of the colony than nearer the margin. It is suggested therefore that, whatever be the cause of the initiation of saltation and in whatever part of the culture the saltant primordia are laid down, a further period of growth is required before they develop to such an extent that they are likely to be picked up when reculture of the fungus takes place. The parent form, so to speak, has a start of the saltant. If, therefore, the medium is such that growth within the body of the colony soon stops, any saltant primordia would be less likely to develop and produce a small region of saltated tissue. The behaviour of a medium such as Richards's solution would be the converse to this. Here there is a concentrated medium with the minimal staling

reactions. On this account strong growth continues over a considerable time, and thus the conditions are optimal for the further growth of saltant primordia. It is suggested, therefore, that saltants are most likely to be formed on a medium which combines high concentration with minimal staling capacity, and conversely that saltants will not be formed freely on a medium in which growth is soon stopped, either by exhaustion of the nutrient or by staling factors. For the purpose of preserving labile forms in culture, the writer tentatively puts forward the following suggestions: (1) to use a very dilute medium (plain agar in many cases would probably be found as serviceable as any other); and (2) to reculture from young cultures, or, better still, to reinoculate from the growing edge of the parent culture. Provided the region of the parent colony behind the growing edge shows the typical appearances of the parent, an inoculum from the edge of the colony will invariably, in the writer's experience, reproduce the parent form. Even with the *Fusarium* strain D, which saltates so freely on Richards's solution agar medium, inocula taken from the edge of the colony have always been found to come up true to type.

The behaviour of cultures of some of these *Fusarium* strains on a medium like Richards's solution indicates a new interpretation of the frequently experienced 'falling off' or attenuation of parasitic fungi on rich media. This is often interpreted in a kind of anthropomorphic way as being a general weakening brought about by over-rich feeding, &c. The results obtained with such a parasitic strain as D throw considerable light on this loss of parasitic power. 'High-feeding' as represented by Richards's solution does not attenuate this strain. The latter can be recovered unaltered from the greater part of the culture, but at the same time pockets of saltant strains appear over the colony, and these are liable to be transferred. Some of these possess feeble parasitic powers or none at all, and the transference of any one of these of course involves the loss of virulence. It is not therefore a case of the loss of parasitic power by the fungus, but rather the loss of the fungus itself.

#### SUMMARY.

1. There is no evidence of slow cumulative change during the culture of the *Fusarium* strains dealt with in this work. Growth curves were determined for a number of strains under standard conditions, and when, after an interval of about a year and a half, the curves were prepared for the same strains under the same conditions, identical results in all essential particulars were obtained.

2. Inocula of spores in the case of some strains give rise to colonies identical with those arising from mycelia inocula. In other cases definite differences do arise in this way, but the difference has always been found to

be small. There is in general no essential difference between spore and mycelial inocula from the point of view of the colonies to which they give rise.

3. Saltations have taken place from time to time in the course of culture of these forms. An account is given, together with photographs, of a number of plates illustrating saltation.

4. Saltation occurs more frequently on some media than on others. Thus transfers to Richards's solution agar in many cases lead to abundant saltation. The new strains do not appear as well-defined sectors in the parent colony, but occur in isolated patches over the surface of the latter.

5. The percentage of saltated areas increases with the age of the colony and is greater in the centre than towards the margin of the colony.

6. A discussion is given of the bearing of these results on the problem of preserving the vigour of strains in culture and suggestions are put forward as to the types of medium to be used, on the one hand for keeping strains in their original form, and on the other for the encouragement of saltations.

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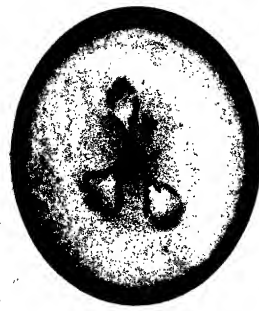
## EXPLANATION OF PLATE IX.

Illustrating Dr. W. Brown's paper on *Fusarium*.

Figs. 1-11. Illustrations of cultures of *Fusarium*, showing saltation. For detailed description see text, pp. 232-4.



4.



3.



2.



1.



6.



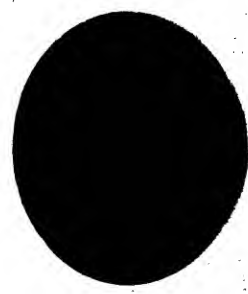
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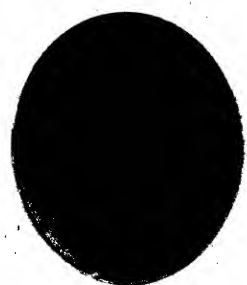
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11.



# The Conditions of Growth and Development of *Pyronema confluens*, Tul. (*P. omphaloides*, (Bull.) Fuckel).

BY

WILFRID ROBINSON, D.Sc.

With four Figures in the Text.

THE work of Klebs (6) on many algae and fungi served to establish the dependence of the sequence of phases in the life-cycle of such plants upon definite external conditions. Broadly speaking, Klebs was led to the conclusion that there exists a clear antithesis between active vegetative growth and the manifestation of reproductive activity, and that in nature as well as in experimental cultures the antithesis depends on the relationship to definite external conditions. Klebs was successful in demonstrating the conditioning factors of the different stages in the life-history of the organisms he studied, and he regarded such external conditions as operating by producing corresponding internal changes which are then manifested in the development of the plant in question. He, however, obtained little precise information regarding the nature of the internal changes which lead to the morphological expression of the effects of external conditions in development. The observations and conclusions of Klebs have been extended by his pupils and later investigators to plants other than those he studied; but we have as yet very little insight into the nature of the internal changes associated with development in fungi.

The occurrence of *Pyronema confluens* in restricted situations in nature, usually on ground which has been burnt, and the ease with which it can be grown and made to carry through its life-cycle under cultural conditions suggested the suitability of the fungus for study from the point of view of developmental physiology. This paper, therefore, deals with the effects of external conditions on *Pyronema* with special reference to the formation of reproductive structures. Before entering into the details of the present work, brief reference may be made to the observations of other investigators bearing upon this question.



R. and C. Tulasne (2) (1861 and 1865) gave some of the earliest descriptions with figures of *Pyronema confluens*, recognizing the reproductive character of the apothecia, but failing at first to discover the significance of the antheridia and oogonia. De Bary (1) (1863) described the sexual organs fully, and then in 1866 the Tulasne (3) brothers observed the fertilization process. Kihlmann (5) (1883) extended our knowledge of *Pyronema* in relation to the development of ascogenous hyphae and made special reference to the occurrence of the fungus on soil which had been burned. In more-recent work on *Pyronema*, Harper (7), Dangeard (9), and Claussen (10) have all been chiefly concerned with the cytology in relation to fertilization and the subsequent development of the ascogenous hyphae and asci in the apothecia.

Kosaroff (8) carried out experiments to determine the cause of the constant appearance of the fungus on soils sterilized by heat and the corresponding absence from unsterilized soils. He showed that heat destroyed substances in the soil capable of inhibiting the growth of the fungus. He states that temperatures from 20° to 30° C. are most favourable for its growth, and he also points out the necessity of light for the development of the apothecia, mentioning that the intensity of the light exercises a modifying influence on the colour of the fungus. Claussen (10) (1912), in describing the methods used by him for the cytological study of the fungus from agar cultures, refers clearly to the necessity of light for the development of reproductive organs, but he does not deal with the matter in detail.

The effect of moisture in the substratum has been considered by various investigators. Van Tieghem (4) found that the size of the sexual organs was greater on relatively moist than on dry substrata, and Harper (7) also states that in nature *Pyromena* is particularly susceptible to drought. Brown (11) also found that the variety *iniseum* described by him was very hygrophytic.

The present work had its starting-point in the confirmation of the fact that *Pyronema* only forms its antheridia and oogonia in the presence of light and in the observation that the subsequent development of the apothecium from the fertilized oogonium up to the maturity of the asci and ascospores is also dependent on light. Simple experiments at once established the fact that not only does light exercise a modifying influence on the depth of colour of the fungus, as had been found by Kosaroff (8), but also that no trace of the characteristic pigment is produced in the dark. This observation led to experiments designed to test as far as possible whether the appearance of the pigment and the development of reproductive organs are causally connected. In order to obtain as much precision as possible in these experiments, a series of nutrition tests was first carried out on similar lines to those of Coons (12). The results of these tests gave data regarding the most suitable synthetic medium for the growth of the fungus. The

chief conditions affecting the development of reproductive organs were then studied in order. Details of experiments on the relation of vegetative growth to reproduction will be described below, and also experiments on the effect of different degrees of humidity on the formation of reproductive structures. Some attempt has been made to study the properties of the pink pigment of the fungus, but, owing to the impossibility of extracting more than very minute quantities of the substance, only simple qualitative and microchemical tests have been possible. The genesis of the pigment in the hyphae has been followed, and it is concluded that its presence is not without significance in connexion with the development of the reproductive structures. The relations between this development and light have been studied by means of artificially illuminated cultures and the dependence of the fungus upon a certain definite amount of light-energy for the production of its sexual organs, and the subsequent development of apothecia has been clearly established by experiments conducted under different light intensities. It has also been shown that the necessary energy can only be obtained from the rays of shorter wave-length in the blue half of the spectrum.

#### *Material, Methods, and Nutrition.*

The fungus was isolated in single spore cultures from material found growing naturally on burnt ground. Cultures were grown on soil-extract agar<sup>1</sup> or upon a modification of Coons' agar, which was found by the nutrition tests described below to be most satisfactory for the growth of the fungus. All the experiments were carried out on subcultures of the fungus obtained from single spore isolations. Throughout, transfers for subcultures have been made by using spores and not pieces of the vegetative mycelium. Although many different isolations were made from material derived from different localities, with the exception noted below,<sup>2</sup> no morphological or physiological differences were detected in any of the strains so isolated. A culture from a single spore carries through its developmental cycle to the ascus and mature ascospore on a suitable medium, if illuminated at a favourable temperature, in from eight to fourteen days. Since it was found early in the work that, as regards reproduction, *Pyronema* is sensitive to the accumulation of even small quantities of CO<sub>2</sub>, the cultures were carried out either in large, loosely plugged, well-aerated tubes, or in Petri dishes 1 in. deep.

Adopting methods similar to those used by Coons in his studies on *Plenodomus fusomaculans*, four series of cultures were started, using as bases stock solutions containing M/5 concentrations of MgSO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>,

<sup>1</sup> This was made up by extracting 200 grm. of ordinary rich greenhouse potting soil with water, making the filtered extract up to 1,000 c.c. and adding 2 per cent. of agar.

<sup>2</sup> In two cases, however, strains originating from single spores were at first apparently normal, but, after many transfers, lost the power of producing pink pigment and reproductive structures. These colourless strains nevertheless maintained the power of vigorous vegetative growth.

$\text{NH}_4\text{NO}_3$ , and of maltose respectively. The series of culture solutions were made up to give the concentrations shown in Table I below, and a fifth series was added in which calcium chloride M/1,000 was used in addition to the other substances in order to determine the effect of the presence of calcium in the nutrient solution. The cultures were carried out in lightly plugged Erlenmeyer flasks of 250 c.c. capacity, and prior to inoculation the flasks and media were steam-sterilized at  $100^\circ \text{C}$ . on three successive days. The inoculations were made with a drop of suspension of spores from a sterile pipette. The cultures were grown at the ordinary temperature of the laboratory (about  $16^\circ \text{C}$ . in the day-time) with its usual fluctuations. They were placed on a bench in the front of a north window. Growth in fluid cultures is much slower than on solid media, but after eleven days mycelium was visible in most of the cultures, and from that day at short intervals detailed notes were made on the appearance of the cultures. These notes are summarized in Table I.

TABLE I.

Composition of Nutrient Fluid.		Culture.	Vegetative Mycelium.	Remarks regarding Reproduction.
$\text{MgSO}_4$ , M/500 $\text{KH}_2\text{PO}_4$ , M/100 $\text{NH}_4\text{NO}_3$ , M/100	} plus Maltose	M/25	A <sub>1</sub> x x x x	No sexual organs or apothecia. No pink colour.
		M/50	A <sub>2</sub> x x x x	No sexual organs or apothecia. No pink colour.
		M/250	A <sub>3</sub> x x x	Hypthal tangles, but no sexual organs.
		M/500	A <sub>4</sub> x x x	Few apothecia on glass.
		M/2500	A <sub>5</sub> x	Very few rudimentary apothecia.
$\text{MgSO}_4$ , M/500 $\text{KH}_2\text{PO}_4$ , M/100 Maltose, M/100	} plus $\text{NH}_4\text{NO}_3$	M/25	B <sub>1</sub> x x x	No mature apothecia, but rudimentary sexual organs on glass.
		M/50	B <sub>2</sub> x x x	Reproductive organs and some apothecia on glass only.
		M/250	B <sub>3</sub> x x x	Reproductive organs and some apothecia on glass only.
		M/500	B <sub>4</sub> x x x	Very abundant on surface of fluid.
		M/2500	B <sub>5</sub> x x x	Moderately abundant crop on surface of fluid.
$\text{MgSO}_4$ , M/500 $\text{NH}_4\text{NO}_3$ , M/100 Maltose, M/100	} plus $\text{KH}_2\text{PO}_4$	M/12.5	C <sub>1</sub> x x x	Sexual organs and young apothecia on surface of glass.
		M/25	C <sub>2</sub> x x x	Sexual organs on glass. No apothecia.
		M/50	C <sub>3</sub> x x x	None on surface, few rudiments on glass.
		M/100	C <sub>4</sub> x x	None.
		M/500	C <sub>5</sub> x x	A few apothecia with mature on glass.
$\text{NH}_4\text{NO}_3$ , M/100 Maltose, M/100 $\text{KH}_2\text{PO}_4$ , M/100	} plus $\text{MgSO}_4$	M/50	D <sub>1</sub> x x x	Sexual organs on glass.
		M/100	D <sub>2</sub> x x x	Few sexual organs on glass.
		M/500	D <sub>3</sub> x x	Abundant sexual organs.
		M/1000	D <sub>4</sub> x	Poor growth. No reproductive organs.
		M/5000	D <sub>5</sub> x	Very poor growth. No reproductive organs.
As in series D, but $\text{CaCl}_2$ , M/1000 in all cases		E		Very abnormal growth, mostly submerged. Sexual organs in one case sparingly on glass.

The relative amounts of vegetative mycelium are indicated thus : x x .

The main conclusion derived from these tests was that in all cases, except in cultures B<sub>4</sub> and B<sub>5</sub>, the concentration of ammonium nitrate was too high to allow of the formation of reproductive organs on the surface of the fluid.

It was found, by testing with diphenylamine dissolved in concentrated sulphuric acid, that in the whole series only the cultures B<sub>4</sub> and B<sub>5</sub>, i. e. with initial concentrations of M/500 and M/2,500 of ammonium nitrate, showed a complete absence of nitrate from the medium after five weeks' growth. It appears, therefore, to be not without significance that the cultures showing this feature should be those in which the most abundant production of apothecia occurred, and that they should be the only cultures in which these structures arose on the surface of the medium. Further evidence will be given below in support of the probability that the development of reproductive structures is definitely related to the exhaustion of the medium in nitrogen. It is seen from Table I that these bodies also appeared in other cultures of series B as well as in the other series where the nitrate was not completely exhausted. This is in all probability explained by the fact that in these latter cases the mycelium grew well out of the fluid over the surface of the glass before forming reproductive structures. In this way the mycelium removed to a distance from the source of nutrient was probably brought into a similar condition, with regard to nitrogen starvation, to that on the surface of the fluid in cultures B<sub>4</sub> and B<sub>5</sub>.<sup>1</sup>

The results obtained in the series D and E showed that these fluids were on the whole unfavourable to the growth and complete reproduction of the fungus. In the series E, in which calcium was present in addition to magnesium, the former ions apparently had some inhibiting action on the vegetative growth of the fungus, for the mycelium in these cultures was much more branched and the cells were much shorter and more swollen than in normal cultures. The effects may have been osmotic, but no measurements of the osmotic values of the solutions were made.

At a later stage in the work the relations between the composition of the medium and the growth and development of the fungus were studied in somewhat greater detail than in the above preliminary experiments. The later experiments may be conveniently described at this stage, before passing to the portions of the paper dealing with the effect of conditions other than those that are purely nutritive in character. Four series of cultures were made in which, as before, the concentrations of maltose, ammonium nitrate, magnesium sulphate, and potassium dihydrogen phosphate were successively varied. It will be seen from Table II that in this series of experi-

<sup>1</sup> It is shown later that the humidity of the atmosphere over a culture is also an important conditioning factor for the development of reproductive organs, but in these cultures, the surface being covered by a mat of fungus and the flask lightly plugged, the humidity was probably considerably below saturation.

ments the concentrations of the nutrients tested were much lower than in the earlier series. Five flasks were made up with media at each of the different concentrations as shown in Table II, 50 c.c. of fluid being used in each case. A trace of ferric chloride was added to the stock solutions from which the cultural fluids were made up. Inoculation was made with a drop of a suspension of spores and the cultures were grown for twenty-three days, the appearance of each being recorded at frequent intervals. At the end of the period the mycelium from each culture was carefully washed with cold water and dried to constant weight at 60° C. It is recognized that the using of such a small number of cultures as five for each measurement increased the possible error due to individual variation, but the probable error was in no case found to be greater than  $\pm 10$  per cent. of the average weight given. The probable error was occasionally high, but only the general trend of the figures is being taken into account. The concentrations used in this series and the results obtained are summarized in Table II.

In each of the series it is seen that the concentration of M/2,000 ammonium nitrate is the most favourable for the production of reproductive structures, and the tests for the presence of nitrate (in the culture fluid after twenty-three days), using diphenylamine and concentrated sulphuric acid, indicated that the initiation and subsequent development of reproductive bodies follow upon the exhaustion of the medium as regards nitrate. Thus, for example, in cultures A<sub>4</sub>, B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>, and all the cultures in series C as well as in D<sub>2</sub>, the presence of antheridia, oogonia, or apothecia is correlated with the exhaustion of the nitrate available in the medium. As regards the dry weights of mycelium obtained in the various series it is of significance that in the series A the dry weights approximately correspond to the concentration of maltose in the medium. In series B, on the other hand, although the concentration of ammonium nitrate varies from M/8,000 to M/125, the average dry weight of mycelium obtained at each concentration is of similar order of magnitude throughout the series.

It appears safe to conclude from these results that the amount of carbohydrate in the culture fluid determines the amount of dry weight of mycelium in a given culture, but that the amount of nitrate available must rise to a certain minimum (approximately M/2,000) before reproductive organs can be formed. Further, these bodies only arise when the available supply of nitrogen is becoming exhausted.

It also appears from these experiments that a necessary minimum of maltose (or of other available carbohydrate) must be present before the development of reproductive structures is possible, yet a point is soon reached at which, with additional carbohydrate, their development does not take place, even though the dry weight attained is much greater than at lower concentrations.

The results of the series C, in which the concentration of potassium

dihydrogen phosphate was varied, indicate that at the concentration used, since the minimum of this substance necessary for the formation of repro-

TABLE II.

Medium.	Culture.	Growth and Reproduction.	Presence of Nitrate.	Average Dry Weight of 5 Cultures. Grm.
$\text{NH}_4\text{NO}_3$ , M/2000 $\text{KH}_2\text{PO}_4$ , M/500 $\text{MgSO}_4$ , M/500	plus Maltose	M/4000 A <sub>1</sub> Very slight growth, few reproductive organs	+	0.00045
		M/2000 A <sub>2</sub> Very slight growth, rather more reproductive organs.	+	0.0007
		M/1000 A <sub>3</sub> Fair growth, few mature apothecia on glass	—	0.0019
		M/500 A <sub>4</sub> Good growth, many mature apothecia on surface	—	0.0032
		M/250 A <sub>5</sub> Very good growth. Many degenerate reproductive organs	—	0.007
		M/125 A <sub>6</sub> Very good growth. Many degenerate reproductive organs	+	0.0049
Maltose, M/500 $\text{KH}_2\text{PO}_4$ , M/500 $\text{MgSO}_4$ , M/500	plus $\text{NH}_4\text{NO}_3$	M/8000 B <sub>1</sub> Small group of rudimentary sexual organs in all flasks	—	0.0033
		M/4000 B <sub>2</sub> Small group of rudimentary sexual organs in all flasks	—	0.0036
		M/2000 B <sub>3</sub> Mature apothecia on surface of fluid in all flasks	—	0.0034
		M/1000 B <sub>4</sub> A few rudiments of reproductive organs	+	0.0034
		M/500 B <sub>5</sub> Very few rudiments only on glass	+	0.0042
		M/250 B <sub>6</sub> No reproductive structures in any flask	+	0.0039
		M/125 P <sub>7</sub> No reproductive structures except on glass in one flask	+	0.0035
$\text{MgSO}_4$ , M/500 $\text{NH}_4\text{NO}_3$ , M/2000 Maltose, M/500	plus $\text{KH}_2\text{PO}_4$	M/2000 C <sub>1</sub> Good mature apothecia in all flasks	—	0.0029
		M/1000 C <sub>2</sub> Good mature apothecia in all flasks	—	0.0025
		M/500 C <sub>3</sub> Good mature apothecia in all flasks	—	0.0027
		M/250 C <sub>4</sub> Good mature apothecia in all flasks	—	0.0029
$\text{KH}_2\text{PO}_4$ , M/500 $\text{NH}_4\text{NO}_3$ , M/2000 Maltose, M/500	plus $\text{MgSO}_4$	M/2000 D <sub>1</sub> Mature apothecia in three flasks	Trace	0.0026
		M/1000 D <sub>2</sub> Mature apothecia in all flasks	—	0.0025
		M/500 D <sub>3</sub> Some mature apothecia, but also abundant degenerate rudiments	—	0.0028
		M/250 D <sub>4</sub> Some mature apothecia, but also abundant degenerate rudiments	Trace	0.0026

ductive structures was available, the dry weight of the mycelium obtained was limited by the amount of maltose available. Similar relations between the weight of the mycelium and the amount of available carbohydrate held in the series D, where the concentration of magnesium sulphate was varied.

Here, however, increasing the concentration of magnesium sulphate above M/500 or diminishing it below M/1,000 tended to render the medium unfavourable for the production of antheridia and oogonia. It will be seen later that these facts are of significance in relation to the origin and development of reproductive structures in plate cultures of this fungus on artificial media.

Results in all essential respects similar to those described above have been obtained in two further sets of cultures in which the concentrations of the various constituents of the medium were successively varied, and the conclusions obtained above have received further confirmation from these additional experiments.

It was thought possible that the chief factor determining the appearance of reproductive structures might be the ratio of the amount of carbohydrate to that of nitrate present in the medium. A series of cultures was therefore made in which, while the absolute amounts, i. e. the concentrations of maltose and ammonium nitrate available, were varied, the ratios of the amounts of these two substances were kept constant.

Five series of four flask cultures, each containing 50 c.c. of nutrient solution, were started by inoculation with a drop of a spore suspension, and the condition of the cultures as regards the production of antheridia and oogonia was observed and noted daily. The results of this experiment are summarized in Table III.

TABLE III.

<i>No. of Series.</i>	<i>Concentration of Maltose.</i>	<i>Concentration of Ammonium Nitrate.</i>	<i>Days for First Sign of Reproductive Organs in all Flasks.</i>	<i>Average Weight of Mycelium in 42 Days.</i>
E	M/100	M/500	Reproductive bodies in one flask only, after 42 days	Grm. 0.033
F	M/250	M/1000	28 days	0.0129
G	M/375	M/1500	20 days	0.009
H	M/500	M/2000	13 to 14 days	0.005
K	M/1000	M/4000	11 days	0.0025

It will be seen from these results that, whilst in all the series the ratio of maltose to ammonium nitrate used, i. e. 4 to 1 (except E, where it was 5 to 1) by molecular proportions, was favourable for the production of reproductive organs and apothecia, these structures did not begin to appear in all the series simultaneously, but their origin showed a definite time sequence dependent upon the absolute amounts of maltose and ammonium nitrate available in the medium. It is very significant, in view of the earlier conclusion respecting the effect of nitrogen starvation on the incidence of

reproduction, that the reproductive bodies should appear first in the least concentrated fluid and take progressively longer to appear as the concentration increased. Their very tardy appearance in cultures of series E and F was to be expected from the earlier results described above, for at these concentrations not only is there still nitrogen available in the nutrient fluid, but the high concentration of maltose, whilst allowing vegetative growth, inhibits the production of antheridia and oogonia. Tests with diphenylamine showed nitrate to be absent from the flasks at the time of the appearance of apothecia, whilst maltose was still present in every case. It is also of interest in this experiment that the average dry weights of the mycelium obtained closely correspond to the amount of maltose available.

It appears from this experiment, as well as from those described earlier, that the most favourable nutritive conditions for the development of reproductive structures are found when the ratio of maltose to ammonium nitrate is about four to one in molecular proportions. There is, however, no doubt that the ratio of nitrogen to carbohydrate available in the medium is not the primary factor determining when the reproductive structures shall arise, but that the dominant factors are the absolute amounts of nitrogen and carbohydrate available. If suitable ratios of these have been present the reproductive bodies arise when the available nitrogen becomes limiting by exhaustion. The results of this experiment thus completely support the earlier conclusion that the reproductive structures begin to appear in a given culture when the supply of nitrogen is becoming exhausted, if at the same time the carbohydrate available for the mycelium is not excessive in amount.

Following on the nutrient tests described above, a solid medium was prepared having the following composition: ammonium nitrate M/2,000, maltose M/500, magnesium sulphate M/500, potassium dihydrogen phosphate M/500, and stiffened with 2 per cent. agar after the addition of a trace of ferric chloride. This medium was used in most of the experiments described below.

### *Hyphal Characters and Growth.*

The characters of the vegetative mycelium and of the branches becoming antheridia and oogonia may be briefly dealt with. When spores of *Pyronema* are sown at the centre of the surface of a nutrient agar in a Petri dish, germination readily takes place at temperatures from 15° C. to 30° C. in about five hours. At 15° C. the mycelium grows very rapidly with rather sparse branching, reaching the margin of a 3½-in. dish in about three days. The mycelium is at first entirely superficial in growth, the hyphae being in close contact with the agar and usually surrounded with a film of moisture.

Little or no aerial mycelium is produced until the margin of the agar surface is reached, and the results given below show that the rate of growth



of the culture, so far as this is measured by the rate of extension across the plate, is accelerating until the margin is reached. At this point, however, there is a sudden diminution in the rate of extension of the hyphal tips, which, nevertheless, usually grow some little distance over the glass. Following upon this arrested growth, lateral branch-systems of hyphae arise in abundance from the mycelium on the surface of the medium. These branch-systems grow away from the surface of the agar into the air, and then branching occurs much more frequently. If the culture is illuminated, groups of antheridia and oogonia develop from the branch-systems in the course of a few hours. The precise time sequence varies somewhat according to the medium used and the condition of the culture as regards transpiration.

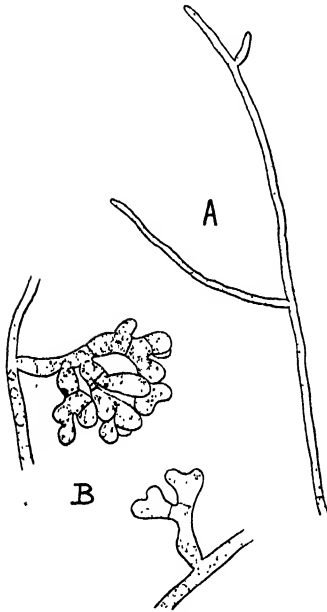


FIG. 1. Hyphae from cultures of *Pyronema confluens* upon agar. A. Extension hypha. B. Lateral branch-systems from which antheridia and oogonia develop.  $\times 225$ .

The first signs of the appearance of antheridia and oogonia are shown by the development of a slight pink colour in the aerial branch-systems and microscopically by the dichotomous branching of the hyphae of the latter. After branching in the air the hyphae do not appreciably elongate, but gradually swell up at the tips to give either antheridia or oogonia. These fertile branch-systems have frequently been figured, e.g. by Tulasne, de Bary, Harper, Dangeard, and Claussen, but Fig. 1 A and B, is given to illustrate the differences between the ordinary hyphae of extension and the reproductive branch-systems.

It will be seen below that these branch-systems or their equivalents are produced both in light and darkness, but that light is neces-

sary for them to develop into reproductive organs; in darkness only certain degenerative structures appear in the place of the latter. The aerial branch-systems are the morphological forerunners of the reproductive organs, and their appearance seems to be conditioned by a check to growth such as is produced when the culture reaches the margin of the medium. The nature of this check to growth will be discussed later. The groups of sexual organs may be confined either to the marginal region of the agar or equally distributed over the surface. The actual distribution in a given culture is determined by the nutritional and other factors dealt with below. It may, however, here be noted that no matter how large the surface of the culture (and dishes up to nine inches in diameter have been used), growth proceeds

rapidly to the margin of the agar surface, antheridia and oogonia never having been obtained until a check to growth has taken place. In this connexion it may be mentioned that on the solid media used *Pyronema* does not show any marked staling effect such as has been found to occur in cultures of certain other fungi.<sup>1</sup> The arrest of growth at the margin of the culture is not a staling effect.

A series of measurements of the rates of growth of different cultures during the course of their development under different conditions has thrown some light on the relations of growth to the reproductive activity of the fungus in culture. These will now be dealt with. Plate cultures were inoculated by the sowing of spores on the surface of the agar in the centre of the plate, and the diameters of the colonies were measured at intervals on successive days. Since it had been found, as stated above, that in Petri dishes the initiation of the development of apothecia by the appearance of sexual organs never took place until the mycelium had reached the margin of the dish, this was definitely controlled by pouring a disc of agar in the central part of each Petri dish. In this way the rate of growth of the hyphae after they left the margin of the agar and grew over the surface of the glass could be measured. The temperature variations in the room in which the experiments were carried out were too slight to affect seriously the rate of growth, but records of the temperature were kept.

Parallel cultures similar in every respect were grown in the light and in the dark and growth measurements taken. In the case of the darkened cultures the advancing hyphae were very briefly illuminated for the few seconds necessary to take the reading. The results of these measurements of rate of extension are shown in Fig. 2, the diameters of the colony being plotted against time.

These curves show that the rate of growth of the fungus in the dark is not materially different from that in the light up to the time when the margin of the agar is reached. In both cases, as would be expected, the growth is suddenly checked at this point. In the case of the illuminated culture apothecial formation is then initiated, and the growth curve continues flat. In the case of the culture grown in the dark, however, after the temporary check, growth-extension continued over the glass, but at a considerably reduced rate. Following on the check to extension operating when the mycelium reached the margin of the agar, the lateral branch-systems of aerial hyphae described above developed both in the cultures in the light and in the darkness. In the cultures in the light these aerial branch-systems give rise to the groups of antheridia and oogonia. In cultures in the dark the aerial branch-systems did not show any of the

<sup>1</sup> In liquid cultures carried on for unusually long periods, i. e. over a month, some amount of staling becomes evident, but in the shorter periods necessary for the appearance of reproductive organs no staling has been detected in the present work.

morphological changes characteristic of the initiation of reproductive organs. Correspondingly there was not the almost complete cessation of growth-extension at the margin which occurs in cultures in the light. The absence of this extension after the onset of reproduction is probably a correlation effect dependent on the utilization of materials available in the hyphae and from the medium, in the processes of reproduction. It is further quite clear that, while the check to growth is immediately followed by the onset of reproduction in cultures in the light, this growth-check is not of itself a stimulus to the formation of antheridia and oogonia, since no such forma-

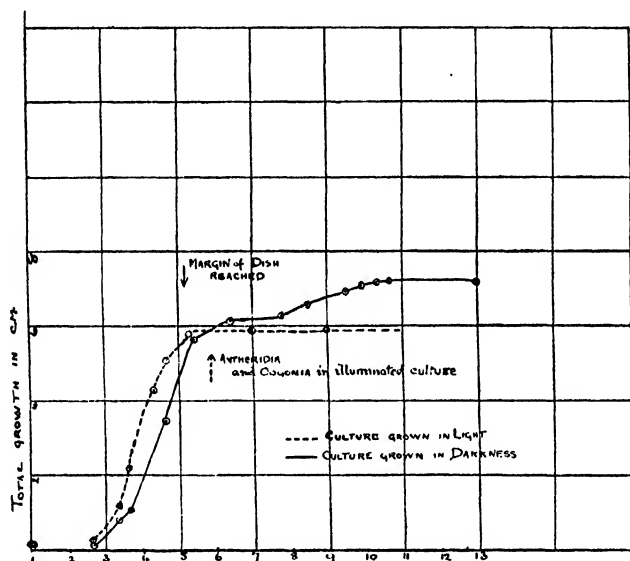


FIG. 2. Curves illustrating superficial growth of *Pyronema* in light and darkness respectively.

tion occurs in the dark. It may, however, operate by bringing the mycelium or substances within it into such a condition that they are susceptible to photochemical changes induced by the light. That this is so was indicated by the following experiment: Two cultures were started at the same time on large agar plates; in the one case the agar was limited to a disc in the centre, and in the other case the medium occupied the whole surface of the plate. The rates of extension were obtained by marking at noon each day the outer limits of the mycelium in each culture, and the average diameter of each culture was recorded. During the night of the fourth day the hyphae reached the margin of the limited disc of agar, and at 5.30 p.m. on the following day the mycelium at the margin was visibly pink, groups of sexual organs being obvious the following morning. In the second culture at the same time there was no visible colour in the mycelium, the margin of the dish had not been reached, and the rate of extension over

the surface was still accelerating. During the night of the seventh day, i. e. three days after the other culture, the margin of the medium was reached by the mycelium, growth-extension was thereby checked, and at 4 p.m. on the following day there was a faint pink colour present in the hyphae at the margin of the agar, and on the morning of the next day groups of sexual organs were visible. The data and curves for this experiment are shown in Fig. 3.

The experiments already described appear to indicate that the check to growth of the mycelium, at the margin of the culture, is a necessary

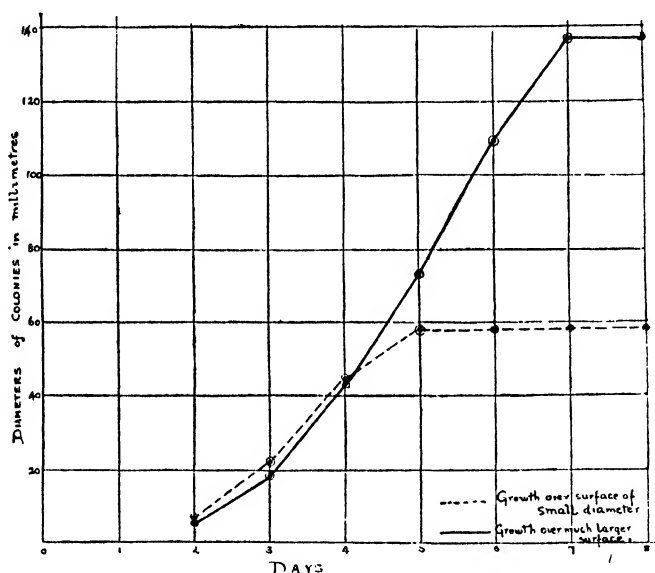


FIG. 3. Curves illustrating the growth of *Pyronema* over surfaces of two different diameters.

preliminary to the initiation of the lateral branch-systems which bear reproductive structures. An attempt, therefore, has been made to analyse, by the further experiments described below, the nature of this check to growth at the margin.

Measurements of the rate of growth were made on cultures in which the method used by Claussen to obtain reproductive structures was adopted. The fungus was allowed to grow from the surface of agar in a small central Petri dish on to agar surrounding it in a very much larger dish (9 inches in diam.). The medium in the central dish contained 5 per cent. inulin, together with M/500 magnesium sulphate, M/500 potassium dihydrogen phosphate, and M/2,000 ammonium nitrate, whilst from the outer dish the inulin was omitted.

Inoculation with spores was made at the centre of the inner dish, and the rate of growth over the surfaces was measured by obtaining the average

diameter of the colony at daily intervals. The results are summarized in curve A of Fig. 4. It is seen that growth proceeded rapidly to the margin of the inner dish; there was then a scarcely perceptible falling off in the rate, growth continuing practically uniformly to the margin of the outer dish, when the real check occurred. A day after this check, antheridia and oogonia were visible in the outer zone of the culture. At the same time as the above experiment, a similar experiment was carried out, using an

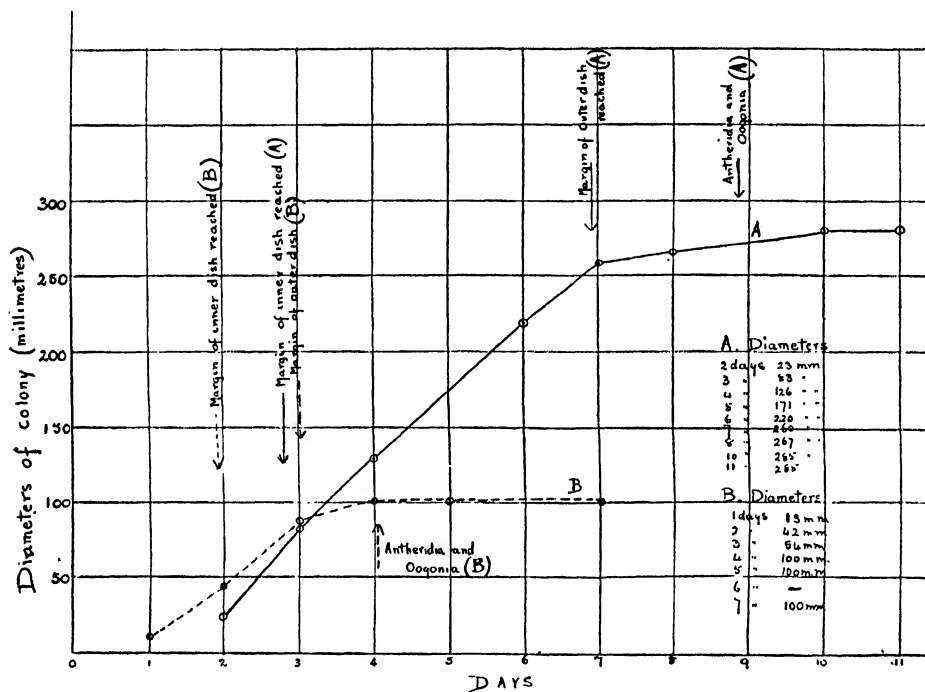


FIG. 4. Curves illustrating growth of *Pyronema* in cultures on agars in double dishes. A. Using large outer dish. B. Using much smaller, outer dish.

outer dish 4 inches in diameter and a smaller central dish. The result, which was similar to that described above, is graphically shown in curve B of Fig. 4.

Many such cultures were made, using Claussen's method of the double dish, but in no case were reproductive structures obtained before the mycelium reached the margin of the outer dish. The absence of carbohydrate from the outer zone of these cultures did not affect growth sufficiently to cause reproductive structures, or the aerial branch-systems bearing them, to appear. The check to growth is evidently not essentially different from that obtained at the margin of cultures on a single medium.

In order to test whether lack of nitrogen at the growing hyphal tips,

after these left the surface of the agar, was the factor conditioning the check, the following experiment was carried out :

A culture was set up in a large dish (9 inches in diam.), with the same medium as before (containing inulin) in the central dish. This was surrounded by a zone of purified agar, which by repeated washing had been rendered as free as possible from nitrates and other nitrogen compounds, the concentration of nitrogen probably being less than M/10,000. The spores were inoculated at the centre of the inner dish. Growth proceeded rapidly to the margin of this inner dish, the mycelium crossed to the outer zone, and then at a slower rate extended to the margin of the outer dish. This growth took over fourteen days, but no sign of reproductive bodies appeared until shortly after the outer margin was reached. Here, however, there was the usual check to growth, and after a few days, i.e. in about three weeks from the starting of the culture, young apothecia appeared on the outer zone of the medium. If lack of nitrogen were capable of directly giving the check to growth, it seems likely that this would have occurred before the hyphae had completed the growth across the zone of agar devoid of any nutrient substance. The result of the experiment rather indicates that, so long as the advancing hyphae are in contact with the moist surface of the agar, supplies of nutrient substances are obtained from the source available in the central dish. When, however, the hyphal tips leave the moist agar surface and grow into the drier air, it seems probable that the lack of moisture at the tips leads to the final check in growth of these.

It has already been mentioned that previous investigators have found *Pyronema* very sensitive in its moisture relations, and experiments on these relations will be described later.

It has been found possible, by a variety of means, to check the growth of cultures before the mycelium has extended to the margin of the agar surface. In one experiment the centre of the surface of the ordinary nutrient agar in a large dish was inoculated. When the fungal colony had about half covered the surface of the agar, the outer, still unoccupied, part of the latter was treated by just touching the surface all round the margin of the dish with a crystal of silver nitrate. The silver salt slowly diffused towards the advancing edge of the mycelium, and in a day checked the growth of this by killing the tips. After three days the uninjured central portions of the colony gave rise to the characteristic aerial branch-systems, which developed normal reproductive structures.

In other experiments the advancing mycelium was checked in growth by forming a narrow ring on the surface of the agar, around the culture, with solutions of lithium chloride and potassium bichromate respectively.

In all such experiments where the tips of the growing hyphae at the outer limits of the fungal mat were injured by chemical means, growth

was checked, and the reproductive structures then appeared and subsequently apothecia were developed.

In another experiment an inner dish was used, containing the ordinary nutrient agar, and the outer dish contained agar to which a considerable amount of lithium chloride was added. This naturally had a considerable drying effect on the agar in the central dish. The mycelium grew to the boundary between the two dishes, but was here checked and did not pass on to the surface of the agar containing the lithium chloride. After two days from the check, normal pink-coloured reproductive organs appeared abundantly over the surface of the central dish. In this, and all the experiments on the growth check, adequate illumination was given by the ordinary daylight of the laboratory.

These experiments confirm the supposition that a check to growth at the margin of a culture of *Pyronema* is an essential preliminary to the development of the lateral branch-systems which give rise to the reproductive structures. It is clear that this check to growth may be produced in a variety of ways. In ordinary plate cultures the passage from the moist agar surface to the relatively dry air leads to greater water-loss from the hyphal extremities, and growth is thereby checked. Where a diffusing ring of silver nitrate is used to check growth and prevent the farther advance of the margin of the colony, the ultimate effect is similar. In this case, however, an actual killing of the tips of the hyphae occurs, but the more profuse lateral branching culminating in the development of aerial reproductive bodies takes place as before. Potassium bichromate acts similarly to silver nitrate in killing the tips of the hyphae. Lithium chloride, on the other hand, has probably a similar effect to dry air, checking the growth by withdrawing water from the tips of the hyphae. It has been shown, in an earlier section of the paper, that the reproductive structures are only developed in fluid cultures under conditions of relative exhaustion of the medium in nutritive substances. In plate cultures this factor also clearly operates, since, if the concentration of either nitrate or sugars be too high, no reproductive structures appear. The invariable absence of apothecia from the central dish in experiments using Claussen's methods illustrates this fact. It should be mentioned, however, that when the ordinary nutrient agar (*vide* p. 253) was placed in the central dish and surrounded by agar of the same composition, except for the omission of any carbohydrate (maltose), all the reproductive bodies appeared on the central dish. Some carbohydrate is evidently required to be available in the medium during reproduction. In Claussen's experiments the high concentration of inulin in the central dish provided a store which was doubtless drawn upon.

The<sup>2</sup> results of the experiments using double dishes, taken in conjunction with those with injurious salts, indicate that the mere growth of the tips on to an agar surface devoid of either nitrate or carbohydrate does not lead

to the radical check to growth which is necessary before antheridia and oogonia appear. A possible direct starvation factor operating locally on the hyphal tips must thus be rejected.

The different water relations of the hyphae in the air, as contrasted with their condition in contact with the moist agar surface, appear the most likely source of the check to growth at the margin of ordinary plate cultures. The experiments with lithium chloride support this explanation, although it has been demonstrated equally clearly that chemical injury to the advancing hyphae, and the provision of an impassable barrier of a poison zone will lead to the same ultimate result as injury by the withdrawal of water. It will be shown below that reproductive structures do not arise in an atmosphere completely saturated with water vapour. In such cultures, however, the hyphae continue to grow at their extremities into the saturated air long after the margin of the dish is reached.

### *Humidity.*

A number of more precise experiments were designed to test the relations of the reproductive activity of the fungus to the moisture in the atmosphere. Cultures were prepared on plates which were then exposed without lids in closed chambers formed by bell-jars. The degree of humidity of the atmosphere was controlled by placing alongside the culture a dish of equal size containing a known concentration of sulphuric acid.<sup>1</sup> By using Regnault's tables for the pressures of aqueous vapour over solutions of sulphuric acid of known strength, concentrations of sulphuric acid were prepared to give relative humidities of 100 per cent., 82.5 per cent., 68.5 per cent., 52 per cent., 30 per cent., and 14 per cent. respectively. The condition of the cultures as regards the production of reproductive organs was noted from time to time, and the results of the experiment are summarized in Table IV. It is of course recognized that, owing to the transpiration of the fungus and the evaporation from the agar surfaces, the method adopted actually gave humidities which only roughly approximated to the figures given. Since, however, only the general trend of the results is taken as significant, and since the simple method does undoubtedly give a range of differing humidities, its adoption appears justified.

It appears that high degrees of humidity of the air over the cultures, as well as low humidities, have an inhibitory effect on the development of reproductive structures by *Pyronema*. The experiments summarized in Table IV indicate that humidities of from 50 per cent. to 70 per cent. probably give the most favourable condition for the production of sexual

<sup>1</sup> It was shown by a preliminary experiment that in an atmosphere controlled by differing concentrations of  $H_2SO_4$  the acid had no deleterious effect on *Pyronema* such as has recently been suggested by Harland to be the case with certain other fungi. An open dish of indicator showed no change in H-ion concentration.



organs and apothecia. That the degree of moisture in the substratum is also of importance in connexion with the reproduction of the fungus has been indicated by some of the experiments described earlier, as well as by previous work on *Pyronema*. A more exact investigation, however, of this relation still remains to be carried out.

TABLE IV.

<i>Relative Humidity.</i> <sup>1</sup>	<i>Condition after 7 Days.</i>	<i>Condition after 9 Days.</i>	<i>Condition after 21 Days.</i>
14 %	No antheridia or oogonia	No antheridia or oogonia	No reproductive bodies.
30 %	Ditto	A very few sexual organs	No mature apothecia.
52 %	Abundant sexual organs	Abundant young apothecia	Second crop of young apothecia.
68.5 %	Signs of sexual organs	Many sexual organs	Many mature apothecia.
82.5 %	No sexual organs	No sexual organs	Considerable number of very small apothecia.
100 %	Only vegetative growth	Only vegetative growth	Only vegetative growth.

#### *Effect of Light on Reproduction.*

The necessity of light as a conditioning factor for the development of reproductive structures in *Pyronema*, first pointed out by Kosaroff, and referred to briefly by Claussen, has been made the subject of special study in the present work. The fact was readily confirmed by growing cultures in absolute darkness, when, even after several weeks, no oogonia or antheridia were ever seen; on the other hand, it was equally readily shown that a relatively short exposure to light of quite low intensity from an electric lamp was sufficient to lead to the normal development of reproductive organs and of apothecia. It was further observed, as a result of preliminary experiments, that the pink pigment characteristic of the reproductive bodies of the fungus invariably accompanies these, and usually makes its appearance in the early development of the hyphal branch-systems from which the sexual organs arise. No trace of the pink pigment has ever been observed in cultures grown in complete darkness. This production of pigment as a concomitant of the early stages in the development of antheridia and oogonia from the hyphae of the branch-systems on which those arise strongly suggested that the pigment itself may play a causal part in the development of the reproductive bodies in relation to the light. Conclusive evidence that this is so has not been obtained from the experiments which will be described below, but the facts so far obtained regarding the development of the pigment and the properties of this, as well as the general relation of the fungus to light, are of considerable interest in this connexion.

<sup>1</sup> The humidities would in all cases be somewhat higher than the percentages given.

It was invariably found that the intensity of light obtained from a north window in mid-winter in Manchester is sufficient to allow cultures to develop sexual organs and normal apothecia. In order, however, to control the illumination of cultures, a series of experiments were carried out in a dark room at approximately constant temperature ( $17^{\circ}\text{C.} \pm 1.5^{\circ}\text{C.}$ ) under artificial illumination, to determine the relations of the development of the fungus, as regards the production of pink pigment and of reproductive bodies, to known intensities of illumination. Spores were sown on slopes of nutrient agar in large tubes (1 in. diam.), and the latter were placed at distances of 50 cm., 100 cm., 150 cm., and 200 cm. from a 100 candle-power metal filament lamp. After three days' continuous illumination pink-coloured groups of sexual organs were visible in the culture nearest to the lamp, and on the following day (i. e. the 4th) these bodies were also present in the remaining three cultures. Whilst there was this slight difference in the time of the appearance of the young apothecia in the culture nearest the lamp, there was no indication that the lowest light intensity was limiting, or even seriously retarding, the development of apothecia in the cultures more remote from the lamp. It is of interest to note that the apothecia developed, for the most part, on the agar, or on the glass of the tube on the side nearest to the source of light, and in the course of ten days attained normal size, producing viable ascospores. The foregoing experiment was repeated, using a small 20 candle-power metal filament lamp instead of the 100 candle-power lamp, but the result was exactly similar to that already given. As before, the tube nearest to the lamp showed the pink-coloured groups of sexual organs in three days, while these features were not present in the remaining culture until the fourth day, but otherwise the development was normal.

These preliminary experiments indicated that the minimum intensity of illumination necessary for the development of apothecia is extremely low, but it appeared at first sight surprising that the times taken for the manifestation of the first signs of reproductive bodies at increasing distances from the lamp showed no considerable increase such as might be expected from the Bunsen-Roscoe Law. The explanation was obtained by a series of more critical experiments in which the cultures were illuminated only after considerable growth had taken place in darkness and the hyphae had almost extended to the margin of the plates. This method was adopted since, as has already been shown above, a check to growth appears to be necessary to bring the mycelium into the state in which it can give rise to the branch-systems that develop antheridia and oogonia. It appeared likely, therefore, that the effect of the absorption of light energy was only manifested if the mycelium was in the necessary condition for the production of the branch-systems induced by the check to growth. In this connexion it is of interest to emphasize that the production of the pink

pigment invariably commences near the tips of the hyphae forming the lateral aerial branch-systems.

The cultures after growth in continuous darkness for the three or four days necessary to allow the mycelium almost to cover the plate were placed at distances of 50 cm., 100 cm., 150 cm., and 200 cm. from a 60 candle-power metal filament lamp, and the time of illumination necessary for the production of the first signs of oogonia and antheridia with pink coloration was noted for the respective distances. Several repetitions of the experiment in this form served to show that the Bunsen-Roscoe law was being obeyed, and comparison with an actinometer placed at the same successive intervals from the lamp showed in a striking way the essential similarity between the behaviour of *Pyronema* in regard to different light intensities and the photochemical effect of the electric light on the sensitive paper of the actinometer. It will be shown below that the rays of the visible spectrum which are effective for the normal development of *Pyronema* are confined to the blue end of the spectrum in the range from  $550\ \mu\mu$  to  $400\ \mu\mu$  wave-length. These are the rays of the visible spectrum which are also most effective in the photochemical reduction of silver salt in the actinometer. The use of this instrument was therefore completely justified in determining the rate of falling off in intensity at increasing distances from the lamp. When precautions were taken to eliminate any reflected light, even though the source of light was not a point, no appreciable deviation from the law of inverse squares could be detected by using the actinometer.

The development of the fungus in the entire absence of light shows certain features which are probably of considerable significance in connexion with the morphogenic effect of light. It has been shown earlier that cultures which are kept in continuous darkness exhibit a check to growth when the margin of the medium is reached, though this effect shows itself somewhat more gradually than in illuminated cultures. This check, however, even in darkness, results in the development of the lateral aerial branch-systems, which in the light become groups of reproductive organs. In darkness, the branch-systems appear as dense white tufts, but the tips of the branches become attenuated rather than swollen as when sexual organs are developing. The white tufts of hyphae soon become densely filled with oily material, the tufts enlarge to form definite rounded bodies, the oily material increases in quantity, the bodies darken in colour and ultimately become hard, resistant black sclerotium-like structures about  $\frac{1}{4}$  mm. in diameter. From the position and the development of these black bodies there is no doubt that they arise from hyphal branch-systems which in illuminated cultures would normally have produced groups of antheridia and oogonia and subsequently apothecia. They are therefore the morphological equivalent of the latter, and their formation strongly suggests that

the reproductive bodies are predetermined in the mycelium at the time of the growth check which results in the development of the aerial lateral branch-systems, but that the photochemical action of light is a necessary phase in the sequence of causation if the branch-systems so produced are to develop into reproductive structures.

In other fungi, which show a dependence on light for the formation of reproductive structures, relations similar to some extent to those seen in *Pyronema* have been described. *Pilobolus microsporus* and certain species of *Coprinus*, for example, only develop normal sporangia or pilei respectively if adequately illuminated, but in the absence of light these fungi are stated to give rise to abortive reproductive structures which fail to develop normally owing to the absence of light (13).

Attempts were repeatedly made to bring about the germination of the dark sclerotium-like bodies referred to above, but these attempts were without success, and all the appearances suggested that in the absence of light the branch-systems which otherwise would have become apothecia slowly undergo degenerative changes resulting first in the production of large quantities of fatty or oily material, and then the black pigment, and finally culminating in the death of the whole structures. It is of interest to mention here that cultures of the fungus on media rich in sugar, such as malt-extract agar or prune agar, even when illuminated by daylight, gave no sexual organs or apothecia in spite of vigorous vegetative growth, but in such cultures ultimately after some months of growth large quantities of the black bodies referred to appeared. That the mycelium of such cultures was capable of producing normal reproductive bodies was demonstrated by transferring fragments of this mycelium to favourable media, when apothecia were obtained in the normal time.

In carrying out experiments on the illumination of cultures at different distances from the lamp, it was found that if cultures were allowed to continue growth in the dark for even two days after the margin of the agar was reached, the whitish modifications of the branch-systems referred to above appeared, and then the cultures required a much longer period of illumination before the formation of sexual organs could be induced. If this illumination were long delayed new branch-systems had to develop before any sexual organs appeared.

It was also shown that for cultures illuminated at the most favourable age a short exposure, e. g. six hours at 50 cm. from a 40 candle-power lamp, provided enough energy to allow for the formation of definite groups of oogonia and antheridia, even though the culture was then placed in the dark. Development did not, however, proceed farther unless more illumination was given. A number of other experiments showed that twenty-four hours' illumination at 50 cm. from a 40 candle-power lamp provided sufficient energy to allow of the production of oogonia and antheridia,

and of the development of mature apothecia with normal asci and ascospores capable of germination, even though the culture was grown in continuous darkness except for the period of twenty-four hours' continuous illumination (from the fourth to the fifth day). Although pigment was present the culture was much paler in colour than in normally grown cultures.

From the results of these experiments on the effect of illumination on the development of *Pyronema*, it seems safe to conclude that a definite amount of light energy is necessary for the complete development of the reproductive structures of the fungus, and that this light energy can only be utilized in the production of the reproductive structures if a check to vegetative growth has previously occurred, which check leads to the initiation of potentially reproductive branch-systems. It is noteworthy, however, that light energy falling on the culture shortly *before* the actual check appears to be available for subsequent utilization, whilst, if the light energy is provided later than the growth check, more appears to be necessary to produce the same result as before. The significance of these facts is not altogether clear, but it may be suggested that the effect of the light is to produce a photochemical modification in some substance in the mycelium which arrests the tendency towards fatty and other degenerative changes. These changes can only be arrested if the culture is illuminated relatively early, a more prolonged illumination proving necessary if they have progressed at all.

It is of interest to mention that the reproductive structures only arise on the directly illuminated parts of the mycelium of cultures. This was shown by enclosing cultures in Petri dishes or tubes in black paper and admitting light only to a small limited area of the culture. In every case the sexual organs and apothecia only developed in the region actually illuminated.

It was hoped that a qualitative study of the properties of the pigment produced by the fungus as well as the rate of respiration of cultures in the light and dark during vegetative growth and reproductive activity would throw some light on these questions, but while some knowledge has been gained of the properties of the pigment the direct part played by this in development (if any) is not yet clear. Similarly, no important differences have been obtained from measurement of the respiratory activity of the fungus in the light and dark respectively, such as might have been expected if the pigment was respiratory in character.

### *Effect of Different Regions of the Spectrum.*

The dependence of pigment formation and reproductive activity upon energy supplied by light suggested that the effects were photochemical in nature. In order, therefore, to ascertain which portions of the visible spectrum were effective, the relations of the fungus to rays of different

wave-length were tested by using differently coloured screens which cut off known portions of the spectrum. Solutions of potassium bichromate and of ammoniacal copper sulphate were used to cut off the rays of the two halves of the visible spectrum. Cultures placed behind either of these screens in daylight gave approximately the same amount of growth in nine days, but those behind the blue screen in this time showed the characteristic pink coloration in the mycelium and the presence of sexual organs which later developed into normal apothecia. The cultures behind the bichromate screen showed no sexual organs or pink colour nor any sign of apothecia, but behaved exactly as cultures in the dark. It was even found that the minute blackish bodies referred to above were formed after an interval in the cultures behind the bichromate screen. These results were repeatedly confirmed, and screens of ruby glass and of a solution of orange G behaved similarly to the bichromate screen in cutting off all the effective rays. The results were the more striking since the intensity of the rays passing through the blue screen was very much less than that passing the bichromate screen. These experiments, although somewhat crude, showed quite conclusively that the rays at the blue end of the spectrum are responsible for the change in the mycelium of *Pyronema* (when this is otherwise in a suitable condition) leading to the development of the pink pigment and of reproductive organs.<sup>1</sup>

There appears, therefore, from these experiments, little reason to doubt that the changes induced in the mycelium by the rays of the blue half of the spectrum are photochemical in character. It must be admitted, however, that the precise nature of the photochemical changes induced is still quite obscure. The results obtained nevertheless appear to suggest that the effects of light here observed have a definite sequence of chemical causation.

#### *The Properties of the Pigment.*

Whilst in the present work it was found impossible to grow the fungus in the relatively enormous quantities which would be necessary to obtain a sufficient supply of the pigment for an adequate chemical study of this, a small number of qualitative tests were made which at least indicate to some extent its properties. The results of these tests will now be briefly described.

The solubility of the pigment in water, chloroform, and ether was very slight, but a solution was readily obtained in acetone. Warm absolute alcohol gave an extract which when filtered was a clear pink crude solution of the pigment. On evaporating the alcohol or acetone solution on a slide the pigment was recovered apparently unchanged in the form of oily drops.

<sup>1</sup> The possible part played by ultra-violet rays has not been tested directly, but in all the experiments the glass of the dishes and screens and also of the laboratory windows would cut off the greater part of the ultra-violet rays.

In solution in acetone, if kept in the dark, the pigment remained apparently almost unchanged for a few months, but if exposed to light for a few days the solution was bleached and no pigment could be recovered by evaporation. The pigment was unaffected in colour by dilute hydrochloric or sulphuric acids or even by strong sulphuric acid. Neither was it affected in colour by treatment with a dilute solution of sodium hydrate. These tests and the insolubility in water indicated that the pigment is not an anthocyanin. When it was dried on the slide and treated with strong sulphuric acid there was no immediate change in colour or development of blue coloration as would be expected from a carotinoid pigment. Dilute nitric acid, however, was found to destroy the colour immediately. Similarly, bleaching powder destroyed the colour in the course of a few minutes, and hydrogen peroxide produced a similar change in sixteen hours. The pigment is thus susceptible to the action of light and of oxidizing agents, but treatment with reducing agents such as sodium sulphite and hydrochloric acid or the bisulphite formalin compound gave no change in colour even after forty-eight hours.

#### *Effect of Carbon Dioxide.*

It has been mentioned earlier that cultures were usually grown in wide tubes lightly plugged with cotton-wool or in deep Petri dishes with good air circulation, because carbon dioxide exercises an unfavourable influence on the development of the reproductive bodies. In order to test whether this effect was really due to the deleterious influence of carbon dioxide or the absence of oxygen in tightly plugged tubes the following experiment was carried out:

A number of tube cultures were plugged with cotton-wool, the plugs were pushed down the neck of the tubes, and tightly fitting corks were inserted and sealed with paraffin wax. As controls a set of tube cultures were lightly plugged. Into one half of the corked tubes a small tube of caustic potash was inserted to absorb the carbon dioxide of respiration. All the cultures were started at the same time by inoculation with spores of *Pyronema*, and all were placed in the light of the window on the laboratory table.

After seven days, the control tubes all showed an abundant crop of pink apothecia. Similarly, the set of corked tubes in which the  $\text{CO}_2$  of respiration was absorbed by potash also showed an abundance of pink apothecia in every tube. The original supply of oxygen in the tubes thus amply sufficed for the complete development of the fungus. The remaining set of corked tubes in which the carbon dioxide of respiration was allowed to accumulate gave cultures, which, in all cases, showed vegetative vigour equal to that obtained in the other two sets. There was, however, in no case any development of pink pigment or of normal reproductive bodies in the corked tubes. The cultures behaved exactly as if they

had been in the dark. The minute whitish tufts, described in the previous section for cultures carried out in darkness, made their appearance. These tufts subsequently became black in colour with, as before, an abundant development of oily material. Results similar in every way to those described above were obtained when cultures were grown in an atmosphere to which 5 per cent. of carbon dioxide was added.

The precise significance of the result is difficult to estimate, but it appears possible that the effect of carbon dioxide is to inhibit the photochemical reactions which would have otherwise taken place in the light. These reactions being inhibited, the degenerative changes which ordinarily take place in the absence of light go forward and are manifested just as they are in darkness. In view of this deleterious effect of carbon dioxide it may further be suggested that in addition to supplying the energy for photochemical reactions in the reproductive branch-systems, the light also, by increasing the permeability of the protoplasm of these, facilitates the removal of the carbon dioxide of respiration from the cells.

#### CONCLUSION.

From the experiments described above it appears clear that the growth and development of *Pyronema* are conditioned by a large variety of factors which to some extent interact and are dependent upon one another. Thus, for example, the effect of the absorption of energy from light is only manifested in the development of reproductive organs and apothecia, if the mycelium is in the suitable condition for utilizing this energy.

That this suitable condition is produced by a combination of other factors has become evident. Not only, for instance, must the supplies of nitrogen and carbohydrate have been suitable in amount, but the water relations of the medium and the atmosphere also exercise a determining influence on the growing mycelium. Whilst all the factors are of importance and must be favourable before the reproductive structures can arise, yet a sequence of causation can be recognized.

As a first stage in the sequence we have the definite arrest in growth of the main hyphae of the mycelium, followed by a development of the lateral branch-systems which grow into the air owing to the spacing conditions on the agar surface. Here, obviously, the moisture relations of the hyphae are altered, and the effect of the energy absorbed from light is then manifested in the morphological changes accompanying the development of antheridia and oogonia. The pink pigment appears in the hyphae at the initiation of these changes and increases in amount with development. Whether the appearance of the pigment and the development of the reproductive structures are causally connected has not been definitely established, but the dependence of both upon light and the non-appearance of the



reproductive structures, if some pigment has not first been produced, is significant. It is suggested above that these facts at least indicate that the origin of the pigment may be traced to the same series of photochemical changes which result in the inception of the reproductive bodies.

The aerial branch-systems have the potentiality of developing into reproductive structures before they have received energy from light. This is the explanation of the degenerative structures which arise in darkness in equivalent positions to the apothecia in normal cultures. The relation of light to reproductive activity in this fungus thus operates relatively late upon regions of the mycelium, where the potentiality for development in a definite direction has already been determined. The absorption of a certain amount of energy from light is therefore a final phase in the sequence of causation concerned in the development.

Of the earlier phases of this, the check to vegetative growth at the tips of the extending hyphae seems of greatest importance in leading to the origin of specialized lateral branches. The specific form and lack of vegetative vigour shown by the lateral branch-systems may be connected with the diminution of the supply of nitrogen available to the protoplasm. That this is probable is indicated by the fact that, in liquid cultures, the origin of the reproductive branch-systems is intimately bound up with the relative exhaustion of the nutrient fluid in nitrogen.

In the presence of excess of sugar or other carbohydrate on solid media, though the lateral branch-systems are initiated, only degenerative structures, similar to those produced in cultures in the dark, make their appearance. In some way, therefore, the presence of excess of sugar in the hyphae inhibits the normal effect of light in relation to the development of the pink pigment and of the antheridia and oogonia. Carbon dioxide also exercises a similar inhibitory effect.

The precise modes of operation of the factors which have been recognized of importance in the development of this fungus in the present state of our knowledge can only be a subject for speculation. But it may be suggested that many of the factors, as, for example, moisture or lack of it, excess of carbohydrate or of carbon dioxide, and even the light energy itself, are operating on the growing and developing organism by modifying or conditioning changes brought about by systems of enzymes.

There can be little doubt in the light of published work on other fungi that the zymase system as well as oxidizing enzymes will prove to be present in *Pyronema*. It is also extremely probable that such factors as the concentration of carbohydrate, of water, or of carbon dioxide acting unequally on different enzymes or the substrates of these, would modify the normal course of the changes produced. Or in the case of light the other factors having previously modified the course of such changes in the growing hyphae, further modifications of the complex then occur, as a conse-

quence of which the structures we regard as typical of the organism are produced.

The blackening observed in the absence of light or in the presence of carbon dioxide or excess of carbohydrate indicates abnormal effects of oxidizing enzymes, and it has already been suggested above that light possibly operates by inhibiting such abnormal effects. Such suggestions do not, however, carry us very far towards a real understanding of the processes at work. Only by obtaining much deeper insight than we have at present into the metabolism of fungi in especial relation to development can we hope to obtain light on the more fundamental questions raised by the facts described above for the development of *Pyronema confluens*.

#### SUMMARY.

1. The vegetative growth, development, and reproduction of *Pyronema confluens* are controlled by external conditions. In the present study the effects of nutrition, moisture relations, light, and of carbon dioxide have been analysed experimentally.

2. The most suitable media and concentrations of nutritive materials for the growth and full development of the fungus have been determined by liquid cultures. It is shown that only small concentrations of both nitrate and sugar are necessary for full development, but reproduction only begins in fluid cultures when the available nitrogen is becoming exhausted.

No general development of reproductive structures occurs if the initial concentration of sugar (maltose) in the medium is higher than M/250. In the presence of concentrations of ammonium nitrate from M/4,000 to M/500 and of maltose from M/1000 to M/250, vegetative growth proceeds until the concentrations are reduced to the level at which reproductive bodies can be formed.

3. Reproductive organs of the fungus arise on laterally borne, aerial branch-systems of hyphae. The structures arise in cultures on solid media after the growth of the mycelium over the surface has been checked. In ordinary cultures this is brought about by the growth of the hyphal extremities away from the moist agar surface into the drier air at the margin of the culture. A chemical check locally applied at the boundary of an actively growing culture operates similarly in leading to the change to the reproductive phase.

4. The moisture content of the air over cultures exercises important effects on the development of the reproductive organs. The most favourable conditions for reproduction are found between relative humidities of 50 per cent. and 70 per cent. No antheridia, oogonia, or apothecia can be formed below 15 per cent. or near 100 per cent. relative humidity.

5. Light is an important factor conditioning reproduction in *Pyronema*.

No reproductive structures arise except in the presence of some illumination, although the minimum amount of light energy required is small. The effect of light is quantitative and is proportional to the amount of light energy absorbed. Cultures in the dark give rise laterally to whitish tufts of fine branching hyphae which correspond in position to the fertile branch-systems produced in the light. The hyphae of these tufts have large quantities of oily contents and the tufts later degenerate into dark-coloured bodies.

6. The pink pigment characteristic of the fungus only appears in relation to reproductive activity. It does not develop in darkness nor in the presence of small quantities of carbon dioxide. The appearance of this pigment is probably definitely connected with the photochemical changes involved in the development of reproductive structures. Whether the pigment is merely a necessary by-product of these photochemical changes or is a more essential part of them has not been determined.

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## NOTES.

**A SIMPLE METHOD OF ISOLATING AND HANDLING INDIVIDUAL FUNGAL SPORES AND BACTERIA.**—As our knowledge of fungi and bacteria has progressed it has become increasingly evident that precise information as to the origin of a culture is of very great importance, and this need has shown itself in the devising and adoption of various methods for isolating single spores. But in spite of this demand for a better technique, so far no method appears to have been evolved whose application has had more than a limited scope, and which has been at the same time simple, certain, and speedy in action. It is hoped that the following method will meet many of the difficulties.

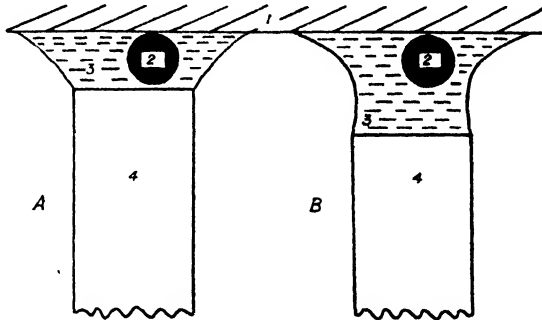
It is obvious that the depth of the film of water on the surface of agar is very small indeed, and is in fact less than the diameter of a bacterium. Consequently when a small number of bacteria are on the surface of agar they will lie side by side and not superimposed one on the other, unless the agar surface is uneven or agglutination has taken place. This being the case, it follows that any means which brings about a local thickening of the surface film will not only allow motile bacteria to show their motility, but will also provide, where the angle between the side of the thickened area and the surface film is sufficient, a means of moving motile and non-motile bacteria and other spores from place to place on the agar surface. In practice, the means employed is a fine glass rod whose diameter is greater than the longer axis of the spore to be isolated, its position as regards the agar surface being capable of fine adjustment in all directions.<sup>1</sup>

In order to watch this process under the higher powers of the microscope, a sterile cover-slip is taken, and a thin layer of agar placed on it. (For work under an oil-immersion lens it is necessary to use cover-slips No. 1, with a thin film of agar obtained by allowing a drop to run down a nearly vertical cover-slip.) In the middle of the agar a few spores or bacteria are placed. The most suitable agar strength has been found to lie between 1·5 and 2·5 per cent.; concentrations below these dry out more rapidly than is convenient. The cover-slip is then placed on the top of a van Tieghem drop-cell, agar downwards, and the rod brought into position through an opening in the wall of the cell. The essential working distance between the objective and the condenser is about  $\frac{1}{8}$  inch. For oil and water lenses it is necessary to attach the cover-slip to the ring with vaseline.

The cover-slip being in position, the bacterial mass is focused under a low-power lens, and its edge brought to the centre of the field. The rod is then moved into position just below the edge of the mass. A high-power lens is now brought into use, and the rod is allowed to touch the edge of the bacterial mass. At once a cone of water is formed between the rod and the agar, some of the bacteria moving

<sup>1</sup> An apparatus has been devised for this purpose and is made by Messrs. Ogilvy, 20 Mortimer Street, London, W. 1.

into it (see A). The rod is then moved away from the bacterial mass, and the bacteria are seen to drop out singly and in groups along the path of the rod. As soon as one is noticed at a slight distance from the rest, the cone is broken by withdrawing the rod. Having moved the rod close to this single bacterium, it is again brought into contact with the water film, but in this case after contact is established the rod is withdrawn from the agar a little so as to form a cone as is shown in B. This cone is passed over the single bacterium and then to the edge of the agar film either by moving the rod, or, by means of a mechanical stage, the cover-slip. The bacterium remains in the cone until this is broken or assumes the shape as in A through some slight alteration in the thickness of the



A. Section showing position when the rod first touches the water film. B. Section showing position when the rod has been withdrawn to form a cone. 1. Agar; 2. Spore; 3. Water; 4. Rod.

agar film. The edge of the film being reached, the cone is broken, the position of the bacterium noted, and the piece of agar surrounding it cut off and transferred to the culture medium.

The reason the bacterium is carried along in the cone (see B) is that the direction of the applied force is nearly parallel to the plane of motion. In A the direction of this force is at an angle to the plane of motion, and the surface tension is then not sufficient to overcome the friction between the bacterium and the agar. That the rod does not take with it any spores or bacteria when it is withdrawn is due to the fact that the diameter of the largest sphere that can be withdrawn with the rod is approximately one-third that of the rod.

The agar film obviously must be as even as possible. One disadvantage of the method is that a certain amount of agar is transferred with the spore; this, however, can be reduced to a minimum by cutting out the piece of agar with the spore by means of a glass tube of suitable size, nipping off the end and dropping it into the culture medium. For bacteria it is best to use mica cover-slips and transfer a piece of cover-slip and agar to the culture medium, after sterilizing the upper surface with alcohol.

The method described was worked out in detail at the Phytopathological Laboratory of the Ministry of Agriculture and Fisheries, to whom I wish to express my thanks for their hospitality.

SYDNEY DICKINSON.

**A SIMPLE DEVICE FOR GASEOUS CIRCULATION IN A CLOSED SYSTEM.**—A device for the continuous circulation of gases in a closed system is often required in chemical and plant physiological work. Any pump which will alternately withdraw and return gas to the system can be used, provided some form of valve is available which will allow the passage of air in one direction only. Light glass balls ground to fit the ends of glass tubing can be employed for such a valve; an arrangement of two vessels with the inlet and outlet tubes dipping under mercury is also commonly used. The use of an ordinary piston pump has two objections—(1) the danger of losing some of the gas by leakage, and (2) the necessity for a motor or other engine to drive the pump. A motor-driven circulating pump (of the Rotoplunge type) can, of course, be employed without a separate valve, but such a pump is generally far too large for a small system and the danger of leakage is serious. The use of a rubber blower as a pump also introduces the liability of the passage of carbon dioxide through the rubber.

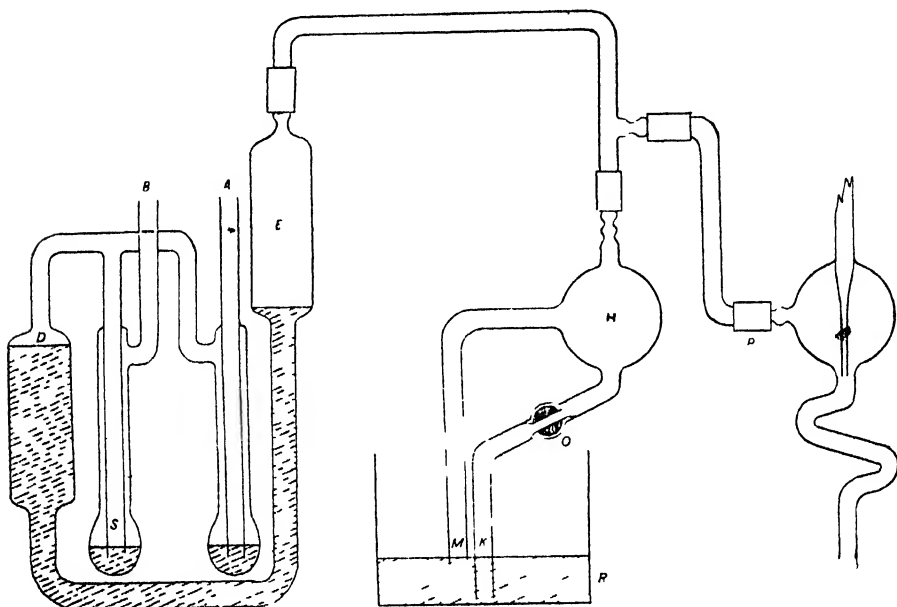
A pump which is available in all laboratories is the ordinary water-pump giving a continuous exhaustion of air. The advantage of the present device is that such a pump is employed, the continuous exhausting action being rendered discontinuous in a very simple manner; furthermore the mercury valve is constructed of glass in one piece and completely seals the system.

The figure on p. 276, which is half actual size, shows the complete apparatus, which is made of glass, the shaded portions representing mercury. On the left is seen the valve system, *D*, *S*, *E*, which allows of a flow of gas in one direction only; on the right is a water-pump, *P*, and in the centre the device, *H*, which converts the continuous action of the pump into a discontinuous one. In use the tubes *A* and *B* are connected with the closed system through which the gas is to be circulated. The water-supply to the pump is adjusted until the right rate of working is obtainable; a finer adjustment may be made by means by a screw clip (not shown) on the rubber tubing at *P*.

When the water-pump is in action the pressure of the air in the vessels *E* and *H* is reduced and the mercury rises both in *E* and in the tube *M*. At the same time the mercury falls in the vessel *D* and gas is drawn into *D* from the system through the 'non-return' valve at the lower end of *A*. The mercury is at the same time being drawn into the bulb *H*, with a resultant fall of the level in *K*. When this fall in level exposes the end of the tube *M*, air rushes in and throws over into *H* the column of mercury in *M*. This mercury falls to the bottom of *H*, and *M* is left in communication with the air, with the result that the negative pressure in *E* disappears. Accordingly the mercury rises again in *D* and the gas is driven out through the 'non-return' valve at the lower end of the tube *S* and back into the other end of the closed system. The mercury in *H* has by this time run back through the tube *K* into the vessel *K*, causing a rise in the mercury level which again seals the end of tube *M*. As the action of the filter-pump is continuous the whole cycle is then repeated.

The circulating device works well and gives very little trouble, the only points of importance in its use being: (*a*) the stopcock *O*, which is used for adjusting the rate of return to the vessel *K*, should have a bore of not less than 4 mm.: (*b*) the mercury vessel *K* should stand in an outer dish surrounded by a cylinder of cardboard, as a certain amount of splashing takes place: (*c*) the bulb *H* should be very firmly

supported in order to withstand the sudden change in weight when the mercury is thrown over : if the support is insufficient the level of the tube *m* changes and the apparatus is put out of adjustment : (d) the bore of tube *m* should be about 8 mm.



Where the chamber through which gases are being circulated contains plants, a tube packed with gold-leaf may be inserted between the end of tube *b* and the chamber ; this should remove traces of mercury vapour. In actual use this gold-leaf has not been found necessary ; no injury to green leaves from the mercury vapour has been demonstrated in experiments lasting over a period of 24 hours.

V. H. BLACKMAN.  
B. D. BOLAS.

# The Pollen Development of *Lathyrus odoratus*.<sup>1</sup>

BY

JOAN LATTER, B.Sc.

With Plates X-XII.

## INTRODUCTION.

**A** CYTOLOGICAL study of *Lathyrus odoratus* was primarily undertaken, in order to determine whether there was any evidence for a physical basis of 'crossing over'. This phenomenon has for many years been recorded in genetical literature, its occurrence in the Sweet Pea being first described in 1909 by Professor Bateson (3), who cited the now well-known behaviour of the linked characters blue flower colour with long pollen and red flower colour with round pollen. Recently Professor R. C. Punnett (45) has published an account of the linkage groups of the Sweet Pea, in which the cross-over values of different characters are stated, and the relative positions of various factors are mapped in certain chromosomes. The exact number of linkage groups has not yet been determined. At present the apparently independent groups are eight in number, but, as the author states, 'the data available are not in all cases sufficient to preclude the possibility of a low grade of linkage between certain of them'. The haploid number of chromosomes in *Lathyrus* is seven, a fact further confirmed by the present work. Professor Morgan and his colleagues, in their researches on *Drosophila*, have shown so conclusively that the number of linkage groups equals that of the haploid number of chromosomes, that we may justifiably hold the opinion of Professor Punnett, 'that the number of linkage groups in *Lathyrus* will eventually be found to correspond to the haploid number of the chromosomes'.

Some interesting cytological observations were made bearing on the phenomenon of crossing over, and subsequently the study was extended to other stages in the pollen development. Particular attention was given to the nuclear activities preceding diakinesis, in the hope of elucidating to some extent the function and fate of the nucleolus, the role of this structure being by no means evident in either plant or animal cytology.

<sup>1</sup> Thesis approved for the Degree of Doctor of Philosophy in the University of London.

(Annals of Botany, Vol. XL. No. CLVIII. April, 1926.)



## MATERIAL AND METHODS.

The material used was of cretin stock, originally obtained from Professor R. C. Punnett. Seeds of both light and dark axilled plants with normal flowers were sown in pots in the Royal Botanic Gardens, Regent's Park, in June 1923, and material collected from the resulting plants in September and October of that year. The plants were allowed to set seed from open pollination. The next generation was sown in May 1924, and material collected from the plants in the following July and August.

Collections of material were usually made between 11.30 a.m. and 1.30 p.m. on bright sunny days. Buds of various sizes were obtained, ranging approximately from 1.5 mm. to 5 mm. in length. Those of greater size were found to have the anthers already yellow, that is, containing fully formed pollen grains. The sepals were removed to allow easy penetration of the tissues by the fixing fluid, and an exhaust pump was always employed.

At the first collection of material two fixatives were used, a 1 per cent. chrome-acetic solution, and Allen's modification of Bouin's fluid (2), given below :

Picric acid, saturated aqueous solution	75 c.c.
Formol (commercial)	25 c.c.
Glacial acetic acid	5 c.c.
Urea crystals	2 grm.
Chromic acid crystals	1.5 grm.

This Bouin's fluid gave excellent results, and in subsequent collections was used for all material to the exclusion of other fixatives. The fluid was heated to an initial temperature of 38° C., and allowed to cool gradually while the collection of material took place. Owing to the fact that the material had to be taken some distance from the Botanic Gardens to the laboratories, it was impossible to keep the exact time of fixation constant for all the material. The variation was approximately between one and three hours. These differences in temperature of fixative and time of fixation had no apparent effect on the results obtained, for all material which subsequently underwent similar treatment showed excellent nuclear and general cytoplasmic fixation. After fixing, the material was run up into 75 per cent. alcohol in one hour, and thoroughly washed in 75 per cent. alcohol to which a few drops of lithium carbonate had been added. After dehydration and clearing, the material was embedded in paraffin, and cut at a thickness of 6, 10, or 12  $\mu$ .

In addition to this cretin stock material, some buds of *Lathyrus odoratus* fixed by Miss E. M. Rees in 1922 in acetic alcohol were also examined.

Various stains were used, including Flemming's triple, Breinl, and Heidenhain's iron-alum haematoxylin, the latter, with or without a counter-stain, giving the best results.

#### RESTING NUCLEI OF THE POLLEN MOTHER-CELLS.

The pollen mother-cells following the last mitosis of the archesporial tissue are distinctly polygonal in outline, and closely packed within the locus. There are no spaces between them, and frequently no space between those at the periphery and the cells of the tapetum. The close contact with the tapetal cells, however, is not constant. The space sometimes observed may be due to contraction of the pollen mother-cells caused by fixation. Very rarely is any shrinkage of the cytoplasm observed at this stage.

The resting nucleus of each pollen mother-cell presents a faintly staining reticulum of granular appearance, and a large deep-staining nucleolus more or less spherical in shape (Pl. X, Fig. 1). This usually occupies the centre of the nucleus. Immediately around the nucleolus there is a comparatively clear zone, traversed occasionally by granular strands of the reticulum, while at the periphery of the nucleus the network is aggregated into a compact pale-staining mass. The strands which lie across the clear zone appear to penetrate the nucleolus, but this could not be definitely determined on account of the deep-staining character of the nucleolus relative to that of the reticulum. Sometimes two and occasionally three nucleoli were observed in a single resting nucleus. If more than one were present, the individuals were of smaller size than the more commonly occurring single structure.

Certain preparations of the resting stage were faintly stained in order to examine the nucleolar contents. The peripheral region of the nucleolus has a greater staining power than the central area, thus giving the appearance of a large central vacuole. In many cases this pale-staining area is distinctly polygonal in shape, showing four or more flat faces.

In addition to this apparent vacuole, and deposited within it, is a small crystal-like structure. Occasionally two or more such bodies are present in a single nucleolus. These bodies vary considerably in shape, appearing oblong, triangular, or almost spherical, and all stain deeply with the reagents employed. Preparations which were favourably stained for observation of the nucleolar contents showed these depositions to be an almost constant feature of the nucleoli. If, however, differentiation of the stain had been very prolonged, with the result that the nucleoli were almost decolorized, no such bodies could be detected in the central vacuolate portion. The fact that they are not observed in these preparations does not prove their absence from the nucleoli. Presumably during differentiation

the stain leaves the crystal-like inclusions more rapidly than it is given up by the peripheral regions of the nucleolar substance, with the result that the faintly stained sections show no sign of these structures. We may, however, justifiably assume them to be a constant feature of each nucleolus, for in those preparations favourably stained for observation of nucleolar inclusions, these crystal bodies have always been found. Similar intranucleolar crystal bodies are found in the tapetal cells at this time (Pl. X, Fig. 2). No nucleolar inclusions of this type can be detected if the nucleoli, which have a great affinity for stains during the early prophase period, are deeply coloured. This may explain the fact that no former record exists of a constant crystal-like inclusion of the nucleolus, though the occasional occurrence of such structures has been noted.

The nature of this crystal body is difficult to determine. Possibly it is a deposition of protein, its appearance being very similar to that of protein crystalloids found in somatic tissues. Its situation within a vacuolate area is a further point of similarity. On account of its extremely small size, specific tests were almost impossible. Slides were treated with 1 per cent. osmic acid, and on examination showed the crystal body to be a lightish brown. The reaction, however, could not be considered as convincing. We may, nevertheless, conclude that the body is not of a fatty nature, as no intense stain was caused by the osmic acid. Unsuccessful attempts at specific staining were also made with Millon's reagent and nitric acid.

Similar structures have been observed in the resting nuclei of the pollen mother-cells of *Oenothera franciscana* (8). In this form the 'crystalloid bodies' are occasionally found in the vacuolated areas of the nucleoli. Miss Digby (12) describes 'crystalline-looking bodies' occurring in the outer cells of the root of *Galtonia candicans*. In these cells she observes one or more such 'structures' present in the resting nuclei, these apparently originating in the nucleolus. They therefore differ somewhat from the crystal bodies of *Lathyrus*, which were never found free in the nuclei, but always embedded in the nucleolus.

#### HETEROTYPIC PROPHASE.

*Synizesis.* The first indication of approaching prophase is shown by the contraction of the reticulum away from the nuclear membrane. This leaves a perfectly clear zone surrounding the contracted mass, in the middle of which lies the embedded nucleolus (Pl. X, Fig. 3). This clear zone was proved by measurements to be due to contraction of the reticulum and not to membrane expansion, which, however, takes place later.

The large nucleolus gradually leaves its central position, and passing through the contracted reticulum comes in contact with the nuclear membrane (Pl. X, Figs. 4, 5). During its passage to the periphery of the nucleus

its spherical shape is lost, and a more elliptical form assumed. When in contact with the membrane the nucleolus becomes flattened along it (Pl. X, Fig. 6), and this flattening process continues till in the late prophase stages the typical crescent shape is apparent. The nucleoli present in any one locus may move in any direction when leaving the centre of the nucleus. There appears to be no relation between the direction of movement of the individual nucleoli, nor does the direction seem influenced in any way by the position of the nucleus in the cell, or by the direction of penetration of the fixing fluid.

While this change in the position of the nucleolus is taking place, there is considerable expansion of the nuclear area. Simultaneously indications of definite thread formation appear in the reticulum. The thread seems to be formed by rearrangement of the granules in linear series. More probably, however, its appearance is due to the deposition of a stainable substance on a formerly unstainable linin thread on which the granules are situated. Small portions of definite thread-like material appear scattered in the granular mass, indicating that the stainable substance is at first distributed irregularly and later becomes uniform along the whole length of thread.

In the preliminary account of this work (36) the probability of the granular appearance of the reticulum being due to differential staining was briefly discussed. The presence of unstainable connexions between the granules must be assumed in order to account for the continuity and individuality of the chromosomes in successive generations, and from this it follows that the basis of the individuality of the chromosomes probably lies in the linin. Evidence in favour of this view is given in the discussion at the end of this paper.

The later prophase stages show clearly that the method of chromosome pairing in *Lathyrus* is typically telosynaptic, but in these phases prior to synizesis no parallelism of threads, that is, association of similar halves of a univalent chromosome, can be detected.

As the nucleolus lies against the nuclear membrane, 'nucleolar budding' frequently occurs (Pl. X, Figs. 7, 8). The extruded portions apparently persist as additional nucleoli, two or more being of common occurrence in the post-synizetic stages. The extrusions may be compared with those found by Miss Digby in *Galtonia candicans* (11), where she describes 'chromatic bodies' which originate as buds in the nucleolus, and, passing out into the cytoplasm, disintegrate there during the open spireme stage. In *Lathyrus* no passage of the nucleolar buds beyond the nuclear area was observed till the heterotypic metaphase, when the total volume of nucleolar material fragments in the cytoplasm.

In addition to 'nucleolar budding', amoeboid forms of the nucleolus are occasionally found (Pl. X, Fig. 9). In these the central part of the nucleolus

is honeycombed with numerous small vacuoles, while deep-staining processes are put forth from the peripheral parts. These processes taper at their distal ends where they appear to penetrate the granular mass which is becoming progressively thread-like, but no definite continuity can be distinguished between the nucleolar material and the delicate thread. The form of such nucleoli, however, strongly suggests that a transference of material may be taking place from the nucleolus to the thread, and this is supported by the fact that during this period the latter structure is becoming definitely thickened.

Somewhat similar conditions have been described by other authors. Wager (52) records the amoeboid condition of the nucleolus in the nuclear division of *Phaseolus* root-tips. This appearance of the nucleolus coincides with the thickening of the nuclear threads, which are in definite continuity with it. Wager considers that transference of nucleolar material to the nuclear thread takes place, and that the nucleolus is directly concerned in the formation of the chromosomes. More recently Van Camp (51) has described and figured similar amoeboid forms in *Clivia miniata*, and states definitely that the nucleolus contributes substance to the formation of the chromosomes. We cannot, however, assume that passage of material from the amoeboid-like processes of the nucleolus is the general method of thread formation in *Lathyrus*, as this type of nucleolus is of rare occurrence.

After the normal nucleoli have assumed an elliptical form and come in contact with the nuclear membrane, the mass of delicate threadwork generally appears to move away from the nucleolus, and takes up its position on the opposite side of the nuclear cavity. The nuclei at this stage show considerable increase in size over those in the resting condition, the average diameters being about  $14\ \mu$  and  $10.5\ \mu$  respectively. The mass of thread still presents a distinctly granular appearance, indicating that the deposition of the deep-staining chromatin has not yet attained uniformity.

The separation of the knot of thread from the nucleolus is never complete. The nucleolus, which lies flattened against the nuclear membrane, is connected to the compact mass of thread by means of a few delicate strands. Soon after separation from the nucleolus, the thread loses its granular character and becomes a definite and apparently continuous structure lying in a tightly coiled knot typical of the synizetic stage. During the period of thread formation there is no evidence of the approximation of similar halves to form the univalent filaments as observed by Miss Digby in *Osmunda* (13). The constant connexion of the thread with the nucleolus during the distribution of deep-staining material along it suggests that transference of chromatin from the nucleolus is taking place.

*Nucleolar contents.* In order to examine the nucleolar contents during the early prophase stages, certain preparations were very faintly stained with various reagents. During the passage of the nucleolus to the peri-

phery of the contracted reticulum, several very small nucleolar inclusions can be detected. Sometimes they are present as well-defined dark-staining 'crystalline structures', at other times as colourless bright-shining 'patches'. Their shapes appear variable, but on account of their minute size these could not be accurately ascertained. Their appearance as dark-staining 'crystalline structures' (Pl. X, Fig. 10) suggests that the larger crystal body of the nucleolus in the resting stage has undergone fragmentation, while their appearance as bright-shining 'patches' suggests that possibly these 'structures' are merely vacuoles: their extremely small size renders an exact determination on this point impossible. A comparison of these 'structures' with the small vacuoles in the nucleolus seen during diakinesis shows that they appear to be of a different nature. The bright 'refractive' character of these minute 'structures' of the nucleolus in early prophase is not apparent in the true vacuoles. When these 'crystalline structures' are present, the former crystal body of the nucleolus has disappeared. This supports the suggestion that they result from its disintegration. Except for these inclusions, the exact nature of which cannot be stated, the nucleolar material is homogeneous throughout, there being no difference in the staining properties of the peripheral and central regions.

As the nucleolus gradually approaches the periphery of the nuclear cavity and frees itself from the contracted reticulate mass, the number of 'crystalline structures' becomes reduced, as though some were dissolving in the nucleolar matrix. The exact point and mode of attachment of the thread to the nucleolus could not be accurately defined, owing to the deep-staining character of the latter structure relative to that of the thread. During the later synizetic phase, however, interesting observations on this point could be made.

During the passage of the synizetic knot away from the nucleolus across the nuclear cavity, it becomes evident that only one 'crystalline structure' persists. In every case one or more loops of thread remain attached to the nucleolus, the apex of at least one loop being directed towards this 'structure'. It is extremely difficult to follow the entire course of a loop where it traverses the nucleolus, as the thread is very fine and not deeply staining. After prolonged detailed examination of these stages, however, it can be seen that one loop of the thread is definitely in contact with the persistent 'crystalline' inclusion (Pl. X, Figs. 11 and 12).

Other nuclei, which present a similar stage of development, show a loop of delicate thread attached to a dark-staining oval body on the periphery of the nucleolus, no crystal-like inclusions being present simultaneously (Pl. X, Fig. 13).

During the loosening of the thread from synizesis, this peripheral dark-staining body becomes considerably larger and very conspicuous in those preparations favourably stained for its observation. Its importance

lies in the fact that the connecting threads which remain in contact with the nucleolus are constantly associated with it. This structure will be referred to as the 'nucleolar body', the term endonucleolus being scarcely applicable since it is apparently superficial in position and is often seen projecting from the nucleolus as a small bud. Except for the presence of this deep-staining nucleolar body, the nucleolus is homogeneous at this stage, there being no distinction between central and peripheral regions.

The origin of the nucleolar body cannot be stated with certainty, but the evidence suggests that it is derived from the crystal body of the resting nucleus. The minute 'crystalline structures', seen in the nucleolus at the initiation of the prophase period, have more of the appearance of fragments of the crystal body than of vacuoles. Slightly later, the thread is seen attached to one of these included 'structures' of the nucleolus, and, as the nuclear development proceeds, a constant association of one loop of thread and the peripheral nucleolar body becomes evident. Transition stages from the persistent 'crystalline structure' to the dark-staining nucleolar body have not been observed. The above-mentioned features, however, together with the fact that there is a complete absence of nucleoli showing both the 'crystalline structure' and the nucleolar body simultaneously, support the view that the nucleolar body originates from the 'crystal-like' deposition of the nucleolus in the resting period. It may be objected that the position of the two bodies in the nucleolus is not identical. Possibly the flattening of the nucleolus along the nuclear membrane would cause the nucleolar body to take up its peripheral position.

In a few cases the synizetic knot does not move away from the nucleolus across the nuclear cavity, but remains closely associated with it. In these, it was impossible to distinguish the nucleolar contents on account of the overlying thread; but, as will be seen from the account of later stages, there is justification for assuming that a constant connexion exists between part of the thread and the nucleolar material.

*Open spireme.* When the thread loosens from synizesis, the knot usually takes up a central position in the nuclear cavity and loops of thread are thrown out in all directions, one loop always maintaining its connexion with the nucleolus. At this period the nucleolus may be in contact with the nuclear membrane for almost three-quarters of the entire circumference of the nucleus. Nucleoli in which extreme flattening of this type has occurred are shown in Pl. X, Fig. 15. Fig. 16 is drawn from a deeply stained preparation, in order to follow the course of the thread more distinctly. The nucleolar contents cannot therefore be distinguished. It will be seen, however, that a portion of the nucleolus projects at the point of contact with the thread. This projection probably marks the position of the nucleolar body. As the loosening of the synizetic knot continues, a typical open spireme is formed, in which the thread lies in large irregular coils throughout the nuclear cavity. At this stage there is no evidence of the

dual nature of the spireme nor of any discontinuity. Small beads of darkly staining substance appear with irregular distribution along the length of the thread (Pl. X, Figs. 17 and 18).

The nuclear area has by now attained its maximum size, the average diameter being nearly  $18\ \mu$ . It was at this period of development that the presence of the nucleolar body was first detected in a faintly stained preparation treated with iron-alum-haematoxylin. The body appears always to lie at the periphery of the nucleolus, usually on the side nearer the nuclear cavity (Pl. X, Fig. 17). Occasionally it is found on the remote side (Pl. X, Fig. 18), and in these cases the more deeply staining thread connected to it can be seen lying across the nucleolus. A considerable enlargement of the nucleolar body takes place prior to this open spireme stage.

Certain nuclei were observed in which the synizetic knot loosens out from a lateral position in the nuclear cavity, instead of lying centrally. The loops are thrown out across the nucleus, reaching away from the nucleolus. Presumably this arrangement occurs in those nuclei in which the synizetic knot persists against the nucleolus. Pl. X, Fig. 19 shows this type of arrangement. The nucleolus is of an irregular shape, with a definite projection at one end to which the thread is attached. The uniform staining of the nucleolus renders it impossible to state definitely whether this projection is the nucleolar body or merely an undifferentiated nucleolar bud. In preparations purposely destined for examination of intranucleolar structures, however, the connexion of the thread to the nucleolar body is so constant that the condition can be assumed to be universal for *Lathyrus odoratus* during the early prophase periods.

Up till now the pollen mother-cells have been distinctly polygonal in outline and closely packed within the loculus. Indications of their separation from one another at the corners become apparent at this stage, but complete rounding off of the cells does not occur. A somewhat angular outline is retained, and the walls of adjacent cells remain contiguous till the development of the microspore nuclei is completed.

*Second Contraction or Brochonema.* When no trace of the synizetic knot remains, the deep-staining thread lies coiled and looped throughout the entire nuclear cavity. The thread is still a perfectly continuous structure, and occasionally shows longitudinal splitting, which, however, is not evident in the majority of nuclei examined. It is the first indication shown in the prophase of the dual nature of the spireme. The following stages, about to be described, clearly point to a telosynaptic interpretation of the method of chromosome pairing. The split observed in the early looping stages must then be regarded as the separation of two halves of a univalent chromosome, which separation is finally consummated on the homotypic spindle. This interpretation, however, receives no support from observations made on the early synizetic stages, for there no evidence is afforded that chromosome



formation is brought about by any general parallelism of threads. Until this looping stage, there is no evidence to show that the spireme is other than univalent in character.

A more definite arrangement of the thread is gradually assumed, and it becomes evident that seven loops are persisting while the others become massed in a central tangle. These central loops appear to be utilized in the organization of those which persist. The appearance does not suggest that there is any actual flow of substance from one part of the thread to another, but rather that each of the smaller loops is shaken out and rearranged so that it forms part of the length of one of the main loops. Continual condensation and contraction of chromatic material is taking place during this period, so that no increase in total length of the main loops results from this rearrangement. As far as can be determined, the loops are all continuous one with another at this stage. The deep-staining character of the central mass of thread, or occasionally of a central nucleolus, renders accurate observations of the loops in this region impossible (Pl. X, Figs. 20 and 21).

The correspondence in the number of persistent loops and that of the haploid chromosomes is recognized at an earlier prophase stage than has previously been recorded in other plant forms. When the seven main loops first become distinct from the others in the nucleus, very little chromatic condensation has taken place (Pl. X, Fig. 20). The interpretation which is at once suggested by this arrangement of the thread is that each loop is composed of a pair of chromosomes, one maternal and one paternal in origin, which are united end to end at the apex of the loop, that is, at that end which lies nearer the nuclear membrane. This view is proved to be correct by the subsequent behaviour of these loops in diakinesis.

A definite orientation of the loops is assumed as contraction of the spireme continues. The seven which persist usually radiate from the centre of the nucleus, causing the whole to appear as a seven-spoked wheel. Sometimes a small spherical nucleolus occupies the position of the hub. The peripheral nucleolus loses its thin crescent shape and again takes up the more oval form of the early prophase, part of it still remaining in close contact with the nuclear membrane. During this period the connexion between the thread and the nucleolar body seems definitely to be severed. Pl. X, Fig. 22 shows the nucleolar body projecting slightly from the apex of the somewhat conical shaped nucleolus. There is a clear space between it and the loop of the spireme which reaches towards it. That part of the thread nearest the body has a thick and distinctly irregular appearance, suggesting that the connexion may recently have been broken down.

Professor Gates has introduced the term 'brochonema' ( $\beta\rho\acute{o}\chi\omicron\varsigma$  = a loop) to describe this 'looping' stage which corresponds to the 'second contraction' of other forms. The introduction of this term brings the nomenclature of this nuclear condition into line with that first employed in 1900

when the terms 'leptonema' and 'strepsinema' were introduced respectively by von Winiwarter (55) and Dixon (14). The condition of the thread during the brochonema period is to be contrasted with that of the strepsinema stage of Dixon, which occurs in the early thread stages prior to second contraction.

In some of the loops the two arms are seen to be in intimate contact and closely twisted round one another for a considerable length at their proximal ends, that is, the ends nearer the centre of the nucleus (Pl. X, Figs. 22 and 23). The arms become free from one another at a slightly later period (Pl. XI, Fig. 24), and this is followed by definite segmentation of the spireme into pairs of chromosomes. Each chromosome pair is derived from one of the seven radiating loops of the brochonema period. The possible physical basis for the phenomenon of 'crossing over' which the brochonema stage affords will be dealt with in the discussion at the end of the paper.

The segmentation of the spireme is accompanied by rapid condensation and contraction of the chromatin. The thickened bivalents at first appear in a more or less continuous chain, the ends of adjacent chromosome pairs being connected by thin strands of dark-staining material (Fig. 26). The chromosomes themselves are very irregular in outline, and have a spongy or vacuolate appearance which possibly is due to the fixative employed. The complete transverse segmentation between the bivalents occurs across the thin connecting strand of material, but its exact position has not been determined. Presumably these strands are part of the true chromosome substance, and consist of attenuated ends of adjacent chromosomes, as at segmentation they are not cut off and left free in the nuclear cavity, but are drawn into the total composition of one of the formerly connected bivalents.

During segmentation of the spireme, the nucleolus frequently undergoes budding or partial fragmentation (Pl. XI, Figs. 25 and 26), the extruded portions remaining in the nuclear cavity as additional nucleoli. The large peripheral nucleolus gradually rounds itself off, and becomes free from the nuclear membrane. It is now in an extremely vacuolate condition. There may be two or three vacuoles of medium size, or numerous smaller ones giving the nucleolus a honeycombed appearance. No trace of the nucleolar body can be detected in this vacuolate nucleolus, nor in the succeeding stages of nucleolar fragmentation. The body has not been observed later than the brochonema period, but the exact time and method of its disappearance are not yet known.

*Diakinesis.* As the bivalent chromosomes separate from one another, they assume various forms. The two chromosomes of each pair usually remain united end to end for some time after the segmentation of the continuous chain of bivalents. Frequently the two free ends of a pair come together, causing the bivalent to become a ring-shaped structure. These

'rings', when twisted, give the 'figure of eight' forms which are typical of early diakinesis. The ring formation is not assumed by every pair of chromosomes; the two arms of a bivalent may break apart at their original point of union (the apex of the loop seen in the brochonema period), and the two components lie across one another or side by side in close contact. When separation of the bivalents from one another is complete, the irregular outline and somewhat spongy character of the chromosomes is still apparent (Pl. XI, Fig. 27). Chromatin condensation and contraction then appear to take place with great rapidity, giving the chromosomes a firm and definite outline and a greater staining power (Pl. XI, Fig. 28). The bivalents become peripheral in the nuclear cavity, and at this stage show no trace of their former vacuolate character (Pl. XI, Fig. 29).

During diakinesis, the nucleus is bounded by a distinct membrane.

Although intimate contact of the two members of each chromosome pair is effected in various ways, in no case was a split observed between the homologous chromosomes which could bring about exchange of segments and resultant crossing over.

*Spindle formation.* In late diakinesis the nuclear membrane becomes increasingly conspicuous, and is frequently seen as a heavy deep-staining line round the nucleus (Pl. XI, Fig. 30). The sharp and definite outline of the nuclear membrane is then lost, and its edges become obscured as though the substance of the membrane were diffusing into the adjacent cytoplasm and nuclear sap. During this process, the former deep-staining character of the membrane disappears. This 'cloudy' zone of substance resolves itself into a dense mass of fine fibres, closely interwoven and crossing one another, which seems completely to surround the nuclear cavity and enclose within it the chromosomes and nucleoli. Gradually the fibres become more distinct, and from the spherical fibrous sheath one or more conical points of convergent fibres are seen extending into the surrounding cytoplasm. The bivalent chromosomes become centrally grouped in the nucleus, and fibres can be seen in definite contact with them, though the actual mode of attachment cannot be ascertained (Pl. XI, Fig. 31). Certain fibres near the periphery of the nucleus can be traced extending the full length of the nuclear area. Those towards the outside of the sheath become merged indefinitely in the granular cytoplasm, except at those points where 'spindle poles' are being initiated.

The formation of the spindle in *Lathyrus* is apparently rapidly completed, since the early stages are of rare occurrence. The observations indicate that here we have a further example of intranuclear spindle formation.

Very few instances of multipolar spindles have been observed, and here a few words may be said regarding the effect of different fixatives on the first-formed spindle fibres. The material fixed in the modified Bouin solution was not satisfactory for the study of the nuclei at this period, no trace

of spindles prior to the definite bipolar form being found, though the general fixation was good. The spindle sheath and a few tripolar spindles (Pl. XI, Fig. 32) were observed in material treated with acetic alcohol or chrome-acetic fixative.

When the bipolar spindles are formed, the fibres are considerably thicker and more distinct than the individuals of the fibrous sheath. They appear far fewer in number, and are generally seen in contact with a member of the group of bivalent chromosomes, and not lying free from pole to pole (Pl. XI, Figs. 33, 34, and 35).

The history of the nucleolus has not been fully traced during the period of spindle formation. After the occurrence of one or more nucleoli enclosed within the spindle sheath (Pl. XI, Fig. 31), no stages showing nucleoli have been observed until numerous scattered fragments, presumably of nucleolar material, appear in the cytoplasm. These are apparent after the organization of a definite bipolar spindle (Pl. XI, Figs. 33, 34, and 35). Undoubtedly forces are at work which cause rapid fragmentation of the nucleolar material into a number of small globules and the passage of these spherical portions out of the nuclear area into the cytoplasm. Van Camp (51) has observed in detail a similar nucleolar fragmentation in the somatic divisions of *Clivia miniata*. He describes and figures the passage of the nucleolar fragments through the spindle fibres, and their extrusion into the cytoplasm, which frequently takes place via the spindle poles.

#### HETEROTYPIC METAPHASE.

The chromosomes become densely grouped on the equatorial plate of the spindle, generally losing all indication of their bivalent nature at this time. The extent to which the condensation of the bivalents takes place appears to depend in part on the fixative employed. The loss of the bivalent character is especially conspicuous in the chrome-acetic fixed material, this fluid giving a very compact and homogeneous appearance to each chromosome pair. The effects of treatment are not, however, constant; chromosome pairs which distinctly show the bivalency sometimes appear in the same bud in which are found those chromosomes whose bivalent nature is completely obscured (Pl. XI, Figs. 34, 35).

The nuclear area is now scarcely distinguishable from the surrounding cytoplasm, which seems to have encroached upon the zone formerly occupied by the unstainable nuclear sap and completely replaced this substance. This cytoplasm, which surrounds the spindles, is generally less dense than that in the more peripheral parts of the cell.

Scattered in the cytoplasm beyond the spindle fibres are numerous pale-staining globules, probably fragments of the nucleolus. These vary in size. As previously stated, no passage of nucleolar fragments from the

nuclear area to the cytoplasm has been observed. In the preliminary account of this work it was stated that the volume of these fragments appeared to exceed that of the original nucleolus. Several measurements have since been made to compare the volumes of nucleoli in diakinesis with the volumes of nucleolar fragments in the cytoplasm per cell in metaphase. It was found that the volume of the fragments was in every case less than the volume of nucleolar material in any one cell in diakinesis. Further evidence in favour of the view that the fragments are derived from the nucleolus is that a tendency to disintegrate is seen in that structure during diakinesis, and also that the fragments do not appear in the cytoplasm till after the disappearance of a definite nuclear membrane. Favourable comparative evidence is also obtained from Van Camp's observations on the nucleolar behaviour in *Clivia*. These fragments observed in *Lathyrus* eventually dissolve in the cytoplasm.

#### HETEROTYPIC ANAPHASE AND TELOPHASE.

At the approach of anaphase it can be seen that each member of a chromosome pair is attached to a spindle fibre by one end, which is that nearer the pole to which the chromosome is drawn (Pl. XI, Fig. 36). As the two univalents of a pair separate from one another, they are conspicuous on the spindle fibres as two widely opened V-shaped structures. This form is due to the appearance of the homotypic split between the two halves of each dual univalent chromosome, which split corresponds to that occasionally observed in the thread of the open spireme. In preparations taken from material fixed in chrome-acetic fluid the compact appearance of the chromosomes is again evident (Pl. XI, Fig. 37).

In the later anaphase condition the dual nature of the univalent chromosomes cannot be seen (Pl. XI, Fig. 38), but it appears again as the chromosomes reach the poles (Pl. XI, Fig. 39). The spindle fibres disappear when the telophase condition is established, their former position being marked by a barrel-shaped zone of granular striations in the cytoplasm. The chromosomes are now very sharply defined and deep-staining.

*Interkinesis.* Between the first and second meiotic divisions there is no formation of resting daughter nuclei. After the arrival of seven chromosomes at each pole of the heterotypic spindle, nuclear sap is deposited around each group, the members of which then take up a peripheral position in the hyaline areas thus formed. The dual nature of each univalent chromosome is very evident during this period, for on arriving at the spindle poles, the two halves of each chromosome separate from one another at both ends, remaining attached only at the centre. One half-chromosome of each univalent swings round and lies across the other approximately at right angles to it, causing the whole to appear as a cross (Pl. XI, Fig. 40). Considerable

elongation of the chromosomes occurs during interkinesis (Pl. XI, Fig. 41), and anastomosing strands may be seen between them (Pl. XI, Fig. 42). Although these connexions occur between the chromosomes, there is no resemblance to a resting reticulum, for the individual univalents can always be distinguished. During this later stage, a nuclear membrane can usually be detected bounding the area of the nuclear sap.

Nucleoli are not formed between the two nuclear divisions, though dark-staining somewhat globular masses are seen in contact with the more faintly staining chromosomes (Pl. XI, Fig. 42.) These dark masses are not evident till after the elongation of the chromosome halves and the formation of connecting strands between them, and are not found free in the nuclear sap, but each appears as a swollen portion of the chromosome with which it is in contact. The chromosomes appear to extrude this deep-staining material, which, however, is not completely liberated before the univalents become organized for the homotypic division.

#### HOMOTYPIC DIVISION.

Prior to the formation of the homotypic spindles, the fission in the univalent chromosomes becomes invisible (Pl. XI, Fig. 43). The two halves of each chromosome lie closely along one another, and no evidence of their 'double' nature remains. The connecting strands and nucleolar-like masses disappear, probably being reabsorbed by the chromosomes in preparation for the second division.

The space relationship of the homotypic spindles is very variable; the spindles lying most frequently in the same plane, parallel or at a slight angle to one another. No stage has been found which gives any indication of the origin of these spindle fibres. Probably their formation is similar to that of the heterotypic spindles, as no trace of the nuclear membrane remains after their appearance. The hyaline area formed around the chromosomes during interkinesis persists until the homotypic telophase condition is reached. Occasionally the surrounding cytoplasm encroaches somewhat upon this central region which, however, always remains more hyaline than the peripheral parts of the cell.

The seven elongated univalent chromosomes become grouped on the equatorial plate of the spindles as widely opened V-shaped structures. They are apparently in contact with the fibres at the apex of the V (Pl. XII, Fig. 45).

Relatively few nuclei showing the homotypic anaphase have been observed, this stage, as usual, being rapidly completed. The time at which the chromosome halves separate, and the rate at which they travel towards the poles, appear to vary considerably amongst the individual chromosomes. Two or three members of each group may often be seen

preceding or lagging behind the others (Pl. XII, Fig. 46). Later stages, however, show no evidence that these 'lagging' chromosomes do not eventually enter the chromosomal content of the microspores. No longitudinal split in the chromosome halves is present at this stage.

As the chromosomes reach the poles, the spindle fibres disappear, their position being marked by granular striations in the cytoplasm, similar to those seen after the disappearance of the heterotypic spindles (Pl. XII, Fig. 47). In the early telophase the chromosomes are densely grouped at the poles, and are very deep-staining in character. Gradually this compact formation is broken down, the seven members becoming free from one another, and clear areas of nuclear sap appearing amongst them.

A tetrahedral arrangement of the granddaughter nuclei is usually observed at this stage, though all four nuclei may sometimes occur in one plane. Since the homotypic spindles most frequently lie in the same plane, it would appear that the tetrahedral arrangement of the microspores does not necessarily result from a 'decussate' arrangement of the spindles.

The nuclear area now increases considerably in size, and a membrane is formed delimiting the hyaline sap from the surrounding cytoplasm (Pl. XII, Figs. 49 and 50). The chromosomes of the microspore nuclei rapidly lose their deep-staining character, and anastomose with one another. The appearance of these connexions marks the beginning of the formation of the resting reticulum. The development of this structure takes place rapidly, but during the stages of the process which have been observed there is no indication of a longitudinal split occurring in the individual chromosomes. This observation is in agreement with that made at the heterotypic prophase, where it was noted that in the formation of the spireme there was no approximation of similar halves of threads.

Small nucleoli appear in the granddaughter nuclei simultaneously with the formation of anastomosing strands between the chromosomes. These nucleoli are first formed in contact with the chromosomes, and gradually become liberated into the nuclear sap. They then coalesce to form the one large nucleolus of the resting microspore nucleus.

When the resting condition of the microspore nuclei is fully established, a dark-staining crystal body can be seen deposited within the vacuolate area of the nucleolus (Pl. XII, Fig. 53).

#### POLLEN TETRAD WALL-FORMATION.

When the reduction divisions are completed, the first indication of wall-formation in the cytoplasm of the pollen mother-cell is the appearance of dark-staining zones across the striated areas between the granddaughter nuclei (Pl. XII, Fig. 48). These zones have only occasionally been observed, and are purely temporary structures, having apparently no relation to the

actual process of tetrad wall-formation, which is brought about after their disappearance. The nature of these evanescent cell-plates is not yet known.

Similar transitory structures were observed by Lubimenko and Maige (37) in *Nymphaea alba*, where they appeared in the late anaphase of the homotypic division. C. H. Farr (18) records an 'equatorial streak' resembling a cell-plate occurring after the heterotypic telophase in *Magnolia*, and Gates and Rees (25) have noted an evanescent nuclear plate on the spindle in the heterotypic telophase in *Lactuca*. Possibly this appearance of evanescent cell-plates in certain forms is partly responsible for the rather general assumption, until recently, that the method of tetrad wall-formation in higher plants is by the deposition of a new cell-wall across the spindle as in somatic divisions. It is now found that this method is not universal, but that wall-formation by furrowing occurs in the pollen mother-cells of many forms.

The tetrad walls are formed rapidly in *Lathyrus*, and very few cases of the beginning of wall-formation were found in the preparations examined. It has been noted earlier in this paper that the rounding off of the pollen mother-cells is never completed. These cells retain a somewhat angular outline, and are usually in contact with one or more adjacent cells until the dissolution of the mother-cell walls takes place. At the homotypic anaphase (Pl. XII, Fig. 46), and occasionally at the metaphase, it can be seen that the cytoplasm is separated from the outermost limit of the mother-cell by a wall of considerable thickness. In preparations stained with Heidenhain's haematoxylin alone, this wall is only just discernible, but it shows up distinctly when treated with Delafield's haematoxylin. light green, or gentian violet.

At the reconstitution of the granddaughter nuclei, narrow wedge-shaped grooves are seen extending into the cytoplasm at points midway between them (Pl. XII, Fig. 51). Surrounding the cytoplasm is the greatly thickened wall, which may be seen projecting inwards at points immediately opposite the invaginations in the cytoplasm. Unfortunately the preparations in which this furrowing was observed had been treated only with Heidenhain's haematoxylin. It was therefore impossible to make accurate observations on the deposition of wall substance within the narrow cytoplasmic grooves. The cytoplasm of the mother-cell, which is undergoing invagination, may have contracted away from the thickened wall in certain places. Where this has occurred, it can be seen that the cytoplasm is surrounded by a membrane which appears to extend into the narrow grooves formed, seemingly, by the entry of wedge-shaped masses projecting from the surrounding wall.

\*. Pl. XII, Fig. 52 shows three members of a tetrad completely separated from one another by projections of the thickened wall, which have joined



together at the centre of the tetrad. These projections must have grown inward through the cytoplasm, the process probably taking place by an active secretion from the cytoplasm into the grooves, resulting in the deposition of material at the tips of the wedge-shaped projections, till finally the mother-cell protoplast is separated into four uninucleate microspores, each surrounded by its own membrane.

The origin and nature of this thickening substance have not been investigated. Possibly this thick layer surrounding the protoplast results from active secretion by the pollen mother-cell cytoplasm during the period of nuclear activity, or it may be due to enormous swelling of the original mother-cell wall. The deposition of a similar thick wall has been fully described and figured by Gates (24) in *Lathraea*. He states that the special wall is laid down inside and in contact with the mother-cell wall, but is clearly detachable from the latter, and evidently of different composition. In *Lathyrus* no such evidence has been obtained, for in every case the thickened layer has remained in contact with the original mother-cell wall, which retains its angular outline, and is seen merely as the delimiting layer of the thick wall.

The liberation of the young pollen grains is brought about by the dissolution of the surrounding mother-cell wall and thickened layer (Pl. XII, Fig. 53). A definite wall now surrounds each microspore, resulting from the thickening of the former cell membrane. This wall may be somewhat irregular in outline, the irregularity apparently being due to the formation of the three pores in the pollen-grain wall.

The method of tetrad wall-formation in *Lathyrus* is in accordance with that now known to occur in many of the higher plants. It is not proposed here to consider the literature bearing on the subject, a review of which has been given by Farr (17) up to that date. The work on tetrad wall-formation since that time is discussed by Gates (24) in his detailed account of the process of quadripartition of the pollen mother-cell in *Lathraea*. It appears that this tetrad wall-formation takes place by the method of furrowing in certain Monocotyledons, and in a large number of the Dicotyledons, amongst which number we may now include *Lathyrus*.

#### DEVELOPMENT OF THE TAPETUM.

A striking feature of the tapetum is that its cells remain uninucleate throughout the whole process of pollen development, never assuming the multinucleate character usually apparent in tapetal layers. Very occasionally binucleate cells are observed when the pollen mother-cells are entering synizesis, but the uninucleate condition is almost universal for every cell at every stage of development. The tapetal cells are very large, and often undergo great elongation towards the pollen mother-cells. The protoplast of these elongated cells does not grow correspondingly, but comes to lie

against the inner wall of the cell, the remainder of which is occupied by a large vacuole. Outgrowths of tapetal tissue are occasionally seen projecting into the pollen sac. In one case such an outgrowth was seen reaching right across the loculus, dividing it into two parts. When the fully developed pollen-grains are lying free in the loculus awaiting liberation, the tapetal layer still remains intact, the cells now frequently having undergone arcuate elongation about the axis of the anther.

#### STERILITY.

At various stages in the development of the pollen, certain cells may be seen in an abnormal condition, this usually being apparent during the heterotypic metaphase or later stages. The chromosomes are very vacuolate and pale-staining, possibly on account of a great deficiency in the amount of chromatin present. Frequently, during the heterotypic telophase, the walls of the pollen mother-cells are replaced by numerous dark-staining globules, which seem to be derived from the wall substance. The entire tapetal layer of such loculi, in which this type of disintegration is occurring, is frequently represented only by a mass of these spherical bodies. In the later stages of development, one or more members of a tetrad may become abortive, the remainder retaining a normal healthy appearance. Sometimes no sign of sterility is apparent until the pollen-grains are fully formed; they then become much shrunken and irregular in shape, while the nuclear contents completely disintegrate in the cytoplasm.

A small quantity of material of plants genetically 'sterile' for pollen was examined for comparison with plants genetically 'fertile', such as those described above. Certain abortive conditions are observed in the plants 'sterile' for pollen which are not present in those genetically 'fertile' in this respect. These are an almost complete replacement of the archesporial tissue by the encroachment of the large-celled tapetum, frequent bipartition of the pollen mother-cells after the heterotypic division, and occasional fragmentation of the chromosomes.

Although, as yet, an intensive study of the development of sterile pollen has not been undertaken, the observations so far recorded are not in agreement with those of Gregory (27) made on the abortive development of pollen in sterile plants of *Lathyrus*. He states that sterility is always apparent prior to or during the heterotypic metaphase, and has observed no stages later than this in the sterile forms examined.

The fact that sterility becomes first apparent at so many different stages in the microspore development, and is expressed in such a variety of ways, seems to favour Gregory's idea that the sterility of the male cannot be regarded simply as the expression of a factor for sterility borne in a chromosome, but is rather an expression of some deep-lying phenomenon which affects the physiology of the plant.

## DISCUSSION.

*The Method of Chromosome Pairing.* From the foregoing account it appears that the method of chromosome pairing in *Lathyrus* is telosynaptic and in accordance with the scheme indicated by Farmer and Moore (16). No pairing of threads occurs previous to or after synizesis, but the delicate univalent thread of the synizetic knot gradually contracts into a short and relatively thick structure, simultaneously arranging itself into as many loops as the haploid number of chromosomes. This looped thread is a continuous filament composed of the fourteen somatic chromosomes united end to end, the homologous maternal and paternal chromosomes alternating with one another. The two arms of each loop represent a pair of homologous chromosomes united at the apex of the loop and lying side by side, frequently twisted round one another. This condition corresponds to the second contraction of other forms, and has been termed the brochonema stage. At the segmentation of the spireme, the loops separate from one another, and from each is derived a bivalent chromosome. From this account of the formation of the bivalents it is clear that the pairing of the homologous chromosomes is not brought about during synizesis, and the significance of this condition of the nucleus is not yet appreciated.

It is not proposed here to discuss the differences in the views held by the telosynaptists and parasynaptists, but merely to put forward the evidence afforded by *Lathyrus* supporting telosynaptic interpretation. From the complete absence of thread parallelism, the established relation between the brochonema loops and the bivalent chromosomes, and the linking of the bivalents with one another during the early stages of the segmentation of the spireme, it may be concluded that in *Lathyrus* the chromosomes are united end to end in a univalent spireme.

*Chromosome Number.* Winge (56) published the results of a cytological investigation of *Lathyrus odoratus* which was carried out essentially with a view to ascertaining the chromosome number. The outcome of this work was the definite establishment of the fact that the gametic number of chromosomes is seven, which is further confirmed by the observations recorded above. Winge also finds there is no constant difference in the size of the different chromosomes. In the heterotypic anaphase he observes that the members of one of the chromosome pairs are separated from one another slightly earlier than the remainder, but does not attach any significance to this fact. No evidence of this was obtained by the present author. Another observation made only by Winge is that of the presence of twenty-eight bodies in the heterotypic anaphase, which he interprets as due to the greatly curved form of the univalents and the split occurring between the two halves of each. Such elongated and curved univalents do not occur in the material studied for the present work. The appearance

of chromatin tetrads, as recorded in Winge's Fig. 5, is probably a result of the treatment. In neither account is a split recorded in the chromosomes during the homotypic anaphase.

In comparing these two records of the meiotic divisions of *Lathyrus*, the outstanding fact of importance is that the haploid chromosome number is found to be seven. Prior to the publication of Winge's results, some doubt existed as to whether the number of chromosomes in the haploid condition would be established as seven or eight. Ishikawa (32) in his work, 'A List of the Number of Chromosomes', makes no mention of the chromosome number for any species of *Lathyrus*. It has already been mentioned that the number of apparently independent linkage groups is at present eight. The general opinion, however, is that when further data are obtained from breeding experiments, the number of linkage groups will be found to be seven, which number is now definitely established for the haplophase of *Lathyrus*.

*A Possible Physical Basis of Crossing Over.* The brochonema stage described in the text obviously affords a possible physical basis for the phenomenon of crossing over. From Figs. 22 and 23 it is seen that in a certain number of the loops the two arms are in intimate contact and closely twisted round one another for a considerable distance at their proximal ends (the ends nearer the centre of the nucleus). According to the telosynaptic interpretation, the two arms of any loop represent the two homologous chromosomes of a pair, destined to give rise to a bivalent. This intimate twisting then obviously affords an opportunity for interchange of chromatic segments to take place between the homologous chromosomes. A slightly later stage shows the two arms of each loop free from one another. This may be brought about by a simple untwisting, or by a longitudinal break occurring down the double-twisted portion of a loop, followed by the joining of different segments of the thread at points where they formerly crossed. If the separation of the two arms were brought about by the latter method, segments of thread would be exchanged between the chromosomes of a homologous pair, and crossing over would take place.

No evidence of such a break and re-joining of segments has been obtained. The thread, however, at this stage is still a thin pliable-looking structure, in which exchange of material from one part to another would not appear improbable. This fact, taken in conjunction with the twisting of the two arms of each loop about one another, followed by their separation, strongly suggests that it is in the brochonema stage that the cytological basis for crossing over is to be found.

A somewhat similar condition has been described in the pollen development of *Lactuca* by Gates and Rees (25). The authors here record a typical telosynaptic method of reduction, in which a pachynema thread forms nine loops, each representing a pair of chromosomes attached

end to end. The number of loops observed is that of the haploid chromosomes in *Lactuca*. 'The arms of each loop frequently twist about one another before or after the spireme segments into chromosomes. As these chromosomes condense, they may untwist in some cases, but there is some evidence that they frequently break across the twists, producing a straight line of separation between the two longitudinal halves of a bivalent with crossing over of segments from one chromosome to its mate.' It is thus seen that the looping stage corresponds closely in *Lathyrus* and *Lactuca*, and that the interpretation is the same for each form, though apparently the very definite radiation of the loops observed in *Lathyrus* is not present in both. Definite evidence of a break occurring in the chromosomes resulting in crossing over is obtained from this study of *Lactuca*, and it seems probable that a similar condition may yet be found in *Lathyrus*, which at many stages in its pollen development closely resembles *Lactuca* in chromosome behaviour. The condition of the thread in these two forms differs when separation of the arms of the loops occurs after their intimate twisting. In *Lactuca* this takes place after segmentation of the spireme and during condensation of the chromosomes, whereas in *Lathyrus* both twisting and separation of the arms of the loops occur before segmentation and while the 'chromosomes' are still distinctly thread-like though at times heavily beaded with chromatin globules. This earlier condition of the chromosomes would probably facilitate the exchange of segments, but hinders the detection of a break necessary to bring about the redistribution of parts.

No evidence has been obtained from the material studied that chiasmatty in the sense of Janssens (33, 34) is met with in *Lathyrus*. This observation agrees with that of Winge (56), who also states that in his opinion, in organisms in which breeding experiments have resulted in the view that parts of the chromosomes are exchanged during the reduction division, the process is probably not going on at so late a stage as indicated by Janssens. A similar opinion is expressed by Wilson in his contribution to the critical survey of the chiasmatype theory of Janssens by Wilson and Morgan (54). Wilson states, 'It is, I think, highly probable that the cytological mechanism of crossing over must be sought in some process of torsion and recombination in the earlier stages of meiosis, perhaps during the synaptic phase or slightly later'.

The nuclear activities of *Lathyrus* thus furnish a stage in which this condition is satisfied, for when twisting and intimate contact of homologous chromosomes is evident, the thread is still a delicate structure, having but recently loosened from the synizetic knot. Though there is as yet no evidence of a break which would cause crossing over, from comparative evidence it is probable that the cytological basis of this phenomenon in *Lathyrus* will be found in the brochonema stage.

*The Origin of the Spindle.* Sharp (48) has given a concise review of the chief opinions held as to the origin of the achromatic figure. Early observers looked upon the whole mitotic figure, chromosomes, spindle, and all, as a transformed nucleus, all the structures being formed from the nuclear material at each mitosis. Later, Hermann (29) believed that the spindle originated wholly in the cytoplasm, and, following this, the general opinion was that in the majority of the microsporocytes of the Angiosperms the spindle originates partly from cytoplasmic and partly from nuclear substances.

The evidence afforded by *Lathyrus* is that the spindle is of intranuclear origin. A description of the process of spindle formation has been given in the text, from which it is evident that the fibres seem to be derived from the substance of the nuclear membrane. The manner in which the apparent thickening of the membrane is brought about during diakinesis is not clear. It may be by the addition of substances from the nuclear sap, by the swelling of the original membrane, or by the dense aggregation of minute cytoplasmic inclusions around it. If the thickening is brought about by this latter method, then the spindle fibres would originate from a combination of both cytoplasmic and nuclear materials. There is, however, no evidence that such an aggregation of cytoplasmic particles takes place. If such a movement were occurring in the cell, one would expect a vaguely defined zone of relatively dense cytoplasm to surround the nucleus during early diakinesis. No such differentiation in the cytoplasm is detected. Possibly the employment of a mitochondrial method of fixation would elucidate this point.

Recent investigations on spindle formation have revealed an intranuclear origin for this structure. Devisé (10) gives a detailed account of this phenomenon in *Larix*, where he has proved the spindle to originate from nuclear material. After applying a mitochondrial method of fixation, the author has observed a 'chondriosomal perinuclear zone' formed in the cytoplasm by the dense aggregation of chondriosomes around the nucleus. This zone remains intact during the whole process of division, and after its establishment a dense 'intranuclear peripheral zone' is formed within it, adjacent to the central mass of chromosomes. This 'intranuclear peripheral zone' furnishes the spindle material. Robyns (47) has demonstrated spindle formation in somatic cells by the transformation of nuclear sap. Polar caps are first formed at opposite ends of the contracting nucleus. These caps are of nuclear origin, being evolved entirely in the space formerly occupied by the large prophase nucleus. When the volume of the nucleus diminishes, nuclear sap is extruded and forms the polar caps which are observed projecting into the cytoplasm. These caps give rise directly to the future spindle poles.

A striking observation on spindle formation has recently been made by

Hughes-Schrader (30). In the first maturation division in the egg of *Acroschismus* she observes the formation of a compound intranuclear spindle by the collocation of eight fusiform sheaths of spindle material, each surrounding a chromosome tetrad. These fusiform bodies represent individual intranuclear spindles and are formed in direct association with the chromosome elements. After formation of the fusiform bodies around the tetrads, there is no orientation of the spindles until the eight individual sheaths finally fuse to give rise to the compound bipolar spindle.

From the observations of Devisé and Robyns, supported by those on *Lathyrus* (in which, however, no critical study of spindle formation has been attempted) it would appear that the views of the early workers may ultimately be proved correct, and that, in accordance with their opinions, the spindles of at any rate a considerable number of the higher plants will be found to be of intranuclear origin.

*The Nucleolus.* No attempt will be made to discuss here the vast mass of literature which bears on the problem of the structure and function of the nucleolus. The early work on nucleoli is reviewed by Montgomery (43) in his excellent summary of the whole of the literature up to 1897 dealing with that structure in both animals and plants. Wager (52) in 1904 made a detailed study of the nucleolus in *Phaseolus*, and in his paper included a comprehensive review of the more important works published after 1897 in which the role of the nucleolus is dealt with. The earliest record of nucleolar inclusions is made in 1865 by Schrön; this was followed in 1881 by similar observations of Macfarlane (39) in studying *Spirogyra* and also of Mann (41) working on *Myosurus* in 1892. After consideration of their results, Montgomery states as his opinion that although 'nucleolini' or granules within the nucleolus have frequently been observed, no particular morphological significance can be attached to them. They appear to be only detached portions of the nucleolar substance, and may in some cases be only small vacuoles. No further records of nucleolini occur in the works reviewed by Wager. His own observations, however, on the nuclear divisions in the root-tips of *Phaseolus* reveal the presence of dark-coloured granules in some of the larger nucleoli. To these granules, which he also called nucleolini, he does not attach any morphological significance.

More recently Cleland (8) has recorded an endonucleolus in the pollen mother-cells of *Oenothera franciscana*. This first makes its appearance in early synzesis, and in mid-synzesis shows clearly as a dark spherical body in the pale-staining nucleolus. In the open spireme stage the endonucleolus 'seems quite often to be clearly connected with parts of the spireme', though more usually he finds no attachment of one structure to the other. Cleland also describes an endonucleolus in *Oenothera franciscana sulfurea* (9), but no mention is made of its having any connexion with the spireme. In zoological literature, intranucleolar bodies have been described by Carle-

ton (6) under the name 'nucleolini', as occurring in the columnar epithelial cells of the intestine. These nucleolar inclusions seem of a different nature from those occurring in plant cells, since their division and continuity in successive cell generations has been observed.

From the foregoing account of nuclear events in *Lathyrus*, it is clear that one or more nucleolar inclusions are constantly present during the prophase stages. The true nature of the crystal bodies seen in the nucleolus of the resting pollen mother-cells cannot yet be stated. Evidence does not favour their being crystalline deposits of waste substances such as calcium oxalate. True crystals would give no colour reactions with the staining reagents employed, and, as has been noted in the text, these nucleolar inclusions colour deeply. The oxalate and carbonate of calcium, the most commonly occurring inorganic waste products in plants, are both dissolved by substances present in Allen's modification of Bouin's fluid. It seems most probable that these crystal bodies are composed of a protein food reserve, which might be expected in abundance prior to a period of great activity of the pollen mother-cells and tapetal tissue.

The constant association of the spireme with the nucleolar body during the early prophase stages is a phenomenon not previously recorded in plant or animal cytology. The fact that the time of association between thread and nucleolar body coincides with the time of chromatic thread formation suggests at once that the nucleolar body is of importance in the passage of chromatin on to the linin thread. In the open spireme stage the nucleolar body is considerably larger than in early synizesis. This proves that it cannot be the actual substance of the original nucleolar body alone which is passed on to the thread, and suggests that the function of the body is rather that of an elaborating organ, which transfers the elaborated materials on to the thread with which it is in contact. Further evidence in favour of this view of the role of the nucleolar body is derived from the position of the nucleolus. Soon after the first indication of approaching prophase, the nucleolus moves to the nuclear membrane, and there becomes flattened along the membrane. This position is maintained until the rounding off of the nucleolus in early diakinesis, and suggests an absorption by that structure of substances from the cytoplasm. A flow of material through the nucleolus from the cytoplasm towards the elaborating nucleolar body may cause the homogenous appearance of the nucleolar material at this stage, in contrast with its vacuolate appearance in the resting pollen mother-cell. If the function of this nucleolar inclusion is to elaborate material and pass it on to the thickening spireme, it is natural that it cannot be detected in stages later than the brochonema period, when separation of the thread from the nucleolus takes place. The work of the nucleolar body is then completed. Its fate has not yet been ascertained.

Further problems arise which would be of interest to solve in this con-



nexion. For example, does the nucleolar body remain constantly attached to one definite portion of thread through which chromatin is distributed to the more remote parts, or does the thread actually move round in the nuclear cavity and take up elaborated material from the nucleolar body in passing? Although observations suggest that the latter method is the more probable, the solution of this point, among others, must remain in abeyance pending technique development. Undoubtedly there is a great deal of rearrangement of the coils of thread during prophase, which could give opportunity for the whole length of thread to move past the nucleolar body. Furthermore, the thickening takes place gradually and uniformly. If substances passed from the nucleolar body to one part of the thread only, and from this to the more remote regions, one might expect a considerable thickening of the near parts of the thread before any change was apparent in the further portions. Such a condition is described by Cleland (9). His figures of the pollen mother-cells of *Oenothera franciscana sulfurea* show the greatly swollen appearance of the threads in contact with the nucleolus. If, on the other hand, the nucleolar body is always in contact with only one part of the thread, it would be interesting to know whether in different nuclei the attachment is always to identical chromosomes. In *Lathyrus odoratus*, however, there is no constant difference in size or shape of the chromosomes, and recognition of individual chromatin elements is therefore impossible. If attached to one definite portion of the thread, the nucleolar body is perhaps comparable with the 'polar granules' attached to the ends of the chromosomes in *Phrynotettix magnus* (Wenrich, 53). This comparison could not be carried far, as the nucleolar body in *Lathyrus* is apparently concerned with the organization of the entire chromatic thread, while the 'polar granules' of *Phrynotettix* and associated 'plasmosomes' referred to in other forms by Wenrich appear to bear relation only to single separate individual chromosomes.

Various opinions, not necessary to recount here, have been held regarding the function of the nucleolus. The view which recent cytological work has proved correct for many forms is that the nucleolus contributes material to the chromosomes. This conclusion was first arrived at in 1882 by Flemming (22), and during the next three years his opinion was held also by Strasburger (49) and Guignard (28). Later investigations of Strasburger were opposed to this view, and various functions were assigned to the nucleolus by other observers. Montgomery (43) makes reference to literature in which the nucleolus is recorded to dissolve in the nuclear sap, whence it is taken up from solution by the chromosome filament. In his summary, the author states that although the nucleolus may add substance to the chromosomes during nuclear division, this is not generally considered probable. Wager (52) has shown that the nucleolus is concerned with the formation of the chromosomes, and he records the transference of nucleolar

material to the thread. Cardiff (5) finds in *Acer platanoides* that at the point of contact of linin thread and nucleolus the latter bulges out in a small papilla. In some of the preparations this nucleolar papilla greatly resembles a small escaping vacuole. The same phenomenon has been observed by Cardiff in *Claytonia virginica*. He states that this phenomenon strongly suggests that material flows from the nucleolus to the thread, but his figures do not show a constant connexion of the thread to the papillate projection. Nichols (44) has observed in the developing pollen of *Sarracenia* that globules of material elaborated in the nucleolus escape into the nuclear sap and are absorbed by the linin and distributed along its threads. Cleland (8) points out a great thickening of the spireme threads which are in contact with the nucleolus, and considers that a flow of chromatin from the nucleolus is probably taking place. In 1924 (9) he observes the chromatin leaving the nucleolus and flowing along one or two threads which lead directly to the centre of the synizetic knot. A similar passage of nucleolar material to the spireme is noted by Van Camp (51). The evidence afforded by the present study of *Lathyrus* also leads to the conclusion that the function of the nucleolus is to contribute substance to the formation of the chromosomes.

From the above observations, it would seem that it is the linin framework of the chromosomes, rather than the chromatin, which is of importance in the transmission of hereditary characters. This was first pointed out by Macfarlane (40), who states, 'Undue emphasis has been given to the chromatin granules as the sole bearers of heredity. . . . The linin . . . is itself the bearer in part of hereditary peculiarities.' This idea is again put forward by Nichols (44), who explains the loss in staining capacity of the chromosomes at certain periods, on the assumption that 'the morphological basis of the chromosomes remains in the linin, while that part of their substance which causes them to colour deeply is absorbed by the nucleolus'. On this conception the core of linin would contain the genes, while the material derived from the nucleolus would surround and overlay this, so thickening the thread. Otherwise it must be assumed that there is some orderly arrangement of chromatin in the nucleolus; that it is absorbed from the chromosomes in regular succession at the reconstitution of daughter nuclei, and extruded again in a similar way. It is difficult to see how such an apparently fluid substance in the nucleolus could serve as the physical basis of inheritance of Mendelian characters, and it appears that in order to account for the continuity and individuality of the chromosomes, the morphological basis of these structures must lie in the linin. The chromatin is then regarded merely as a thickening substance which is deposited along the thread immediately preceding nuclear division, altering the acidity of the thread substance and causing it to stain deeply under suitable treatment.

*A Possible Explanation of certain Nuclear Events observed in Lathyrus, especially concerning the Nucleolus.* To account for growth in the nuclear volume, it must be assumed that substances are continually entering the nucleus from the cytoplasm. Brown (4), working on fertilization in *Peperomia sintenisii*, observed that all the essential constituents of cytoplasm may be converted into nuclear sap. On the assumption that this fact is correct, and that this property of cytoplasm is not peculiar to *Peperomia*, the following suggestions are put forward to explain certain observations recorded in *Lathyrus odoratus*. The condition of the nucleus during the prophase period will first be briefly recalled.

In the resting nucleus the nucleolus is central, has a somewhat vacuolate appearance, and contains a crystal body. While the nucleolus is passing towards the periphery of the nucleus, the nuclear volume increases by entry of substance from the surrounding cytoplasm. In early synizesis the nucleolus becomes flattened along the nuclear membrane, and appears homogeneous except for the deep-staining nucleolar body which lies at the periphery. Definite thread formation is apparent in the reticulate mass. If the nucleolar body is derived from the centrally embedded crystal body of the resting nucleus, its peripheral position may be brought about by the flattening of the nucleolus. A certain portion of the inflowing cytoplasmic substances will be absorbed by the flattened surface of the nucleolus in contact with the cytoplasm, and this absorption may cause the disappearance of the nucleolar vacuole at this stage. The thread, during its whole period of organization and thickening, is in contact with the nucleolar body.

It is clear that the fate of the inflowing cytoplasmic substances may be one of two things. If they enter the nuclear cavity direct, they become converted into nuclear sap, but if they enter the nucleolus, other changes are brought about which may convert the cytoplasmic material into 'chromatin'. The nucleolus in diakinesis is always extremely vacuolate, often honey-combed with small vacuoles, sometimes appearing as a mass of bubbles. This suggests that some of the original nucleolar material has been used up during the earlier prophase stages. Possibly it is this material which is added to the cytoplasmic substance and brings about its change into true 'chromatin'. The nucleolar body may function as an area where the mixing and elaboration of the nucleolar and cytoplasmic materials occur, and from which the transference of elaborated substance on to the connected thread takes place.

In certain species of *Amoeba*, *A. glebae* and *A. fluvialis*, Dobell (15) describes the karyosome as consisting of chromatin granules embedded in a plastin matrix. At cell division, these granules form the chromosomes, no other nuclear structure contributing material to their formation. A more extreme case is recorded by Jordan (35) in the eggs of *Echinaster crassispira*. Here the chromosomes arise as direct products of nucleolar

fragmentation, and from no other source. These conditions may perhaps be comparable with that which obtains in *Lathyrus odoratus*, if the 'chromatic substance' in the nucleolus is not located in definite granules as the true 'chromatin' of the chromosomes, but is present in a diffused and modified condition which may be called 'prochromatin'. At the initiation of nuclear division, this 'prochromatin' present in the nucleolus may be utilized in the formation of true 'chromatin', which is transferred to the linin thread and so forms the thickened chromosomes. The vacuolate nucleolus found in the later prophase periods probably consists of the plastin matrix which remains after the abstraction of the 'prochromatin'. Presumably the plastin is chiefly protein.

On consideration of the nuclear events following the reduction divisions, an explanation can be offered of the origin of the nucleoli in the late telophase of the homotypic division.

The thread of the finally organized chromosomes will consist of (1) a linin core and (2) 'chromatin' thickening. This latter is by hypothesis derived from (a) cytoplasmic material, and (b) nucleolar material or 'prochromatin'. In the late telophase, the deep-staining character of the chromosomes is lost, the 'chromatin' breaks down and dissociates from the linin framework which remains a feature of the resting nucleus. As the chromatin disintegrates, it may break up into its original constituents. Since all the essential constituents of cytoplasm are convertible into nuclear sap, the nuclear sap formed in telophase may originate from the cytoplasmic materials present in the composition of the 'chromatin'. The 'prochromatin' would then be liberated, and it seems possible that the daughter nucleoli, which now arise in each nucleus, contain the 'prochromatin' substance of the nucleolus of the pollen mother-cell, that substance having presumably been utilized in chromatin formation.

In the late telophase, a hyaline area of nuclear sap always surrounds the anastomosing chromosomes prior to the appearance of the young nucleoli. If the nuclear sap is in reality derived from the disintegration of the chromatin, the 'prochromatin' being liberated simultaneously, this latter substance must exist for a time in an unstainable condition, becoming evident as globules or young nucleoli at a slightly later period. If the events of the heterotypic metaphase are recalled, a possible explanation of this phenomenon may be found. The remaining portion of the nucleolus, that is, the plastin matrix from which the 'prochromatin' has been absorbed, disintegrates in the spindle-surrounding cytoplasm, where the fragments finally dissolve and perhaps break down into simpler substances. At the reconstitution of the daughter nuclei, the increase in size of the nuclear volume will be brought about by the entry of fluid cytoplasmic materials. In this way the plastin matrix of the nucleolus may pass back in solution into the nucleus, where it unites with the unstainable 'prochromatin', the

combined substances appearing as stainable globules or young nucleoli. If such a recombination of 'prochromatin' and plastin matrix were essential for the acquirement of a staining character by the nucleoli, the delayed appearance of the nucleoli after the deposition of the nuclear sap would be accounted for.

The position in which the young nucleoli arise in the daughter nuclei is variable for different plants, this variability having led to a controversy regarding the substance from which the nucleoli are derived. Montgomery (43) states that in his opinion they are probably of cytoplasmic origin, since they usually arise against the nuclear membrane. The view most generally held is that they are of nuclear origin, appearing either as thickenings on the nuclear membrane, or, as is considered more probable, as extrusions of the chromatin reticulum. The most recent evidence favouring the earlier view of Montgomery is that put forward by Gates (23) from observations on the pollen development of *Oenothera lutea*, where, in the daughter nuclei, several nucleoli appear, frequently attached to the nuclear wall. The suggested origin of the nucleolus in *Lathyrus* brings these conflicting views of intra- or extra-nuclear origin into line. The position in the nucleus at which the nucleoli first appear may depend upon the rate at which the extruded globules of 'prochromatin' are liberated from the parent chromosomes, and may be a factor of small importance in the consideration of the probable substance from which the nucleoli are derived.

#### SUMMARY.

1. The resting nucleus of the pollen mother-cells exhibits a very granular reticulum, in which is embedded a large central nucleolus. Within the vacuolate region of the nucleolus, a crystal body is constantly present, this being probably of proteid nature. Similar structures are found in the tapetal nucleoli.

2. The reticulum contracts from the nuclear membrane at the beginning of prophase, and the central nucleolus passes to the periphery of the contracted mass, which gradually loses its former granular appearance and becomes definitely thread-like. The crystal body appears to undergo fragmentation in the nucleolus.

3. When the nucleolus comes in contact with the nuclear membrane, it becomes flattened along it. Nucleolar budding and amoeboid nucleoli are observed. A single nucleolar inclusion is present at this stage, and to it is attached a delicate strand of the spireme, the rest of which composes the synizetic knot.

4. The synizetic knot moves away from the nucleolus, remaining constantly connected to it by one loop of spireme. The connecting thread is attached to a dark-staining structure at the periphery of the nucleolus. This has been called the *nucleolar body*.

5. The attachment of the thread to the nucleolar body is a constant feature in *Lathyrus*, and has not previously been recorded in plant or animal cytology. Presumably the nucleolar body is derived from the former crystal body, and functions as an elaborating organ which transfers the elaborated material on to the thread with which it is in contact.

6. In the late open spireme stage, seven loops are seen persisting, apparently at the expense of the rest of the spireme. This number corresponds to that of the haploid chromosomes in *Lathyrus*. The correspondence in number of loops and number of chromosomes is recognized at an earlier stage than has been recorded for other plant forms. Occasionally a longitudinal split is seen in the looped thread, which at all stages is a continuous structure.

7. The method of chromosome pairing in *Lathyrus* is telosynaptic. Each of the seven loops represents a pair of homologous chromosomes joined end to end. The spireme is thus composed of the fourteen somatic chromosomes united tandem, the homologous maternal and paternal elements alternating.

8. The term brochonema is applied to the second contraction stage in which the loops radiate from the centre of the nucleus. The two arms of each loop are frequently in intimate contact and twisted round one another, thus affording opportunity for exchange of segments to take place between homologous chromosomes. It is in the brochonema stage that a physical basis for the phenomenon of crossing over may be found in *Lathyrus*.

9. Immediately preceding segmentation of the spireme, the bivalents are seen connected with one another in a continuous chain.

10. In diakinesis, the two members of each bivalent are in intimate contact, but there is no evidence of a break which could cause crossing over. The nucleolus is very vacuolate, and shows a tendency to fragment.

11. The spindle appears to be formed from the substance of the nuclear membrane.

12. After the establishment of the spindles, pale-staining globules, probably fragments of the nucleolus, are seen in the cytoplasm where they eventually dissolve.

13. The heterotypic and homotypic divisions occur normally.

14. No resting nuclei are formed during interkinesis, though anastomosing strands occur between the chromosomes, and dark-staining nucleolar-like bodies are seen in contact with them.

15. During the homotypic division, a thickened wall of homogeneous appearance is deposited round the mother-cell protoplast.

16. After the homotypic telophase, evanescent cell-plates may occur in the cytoplasm in the positions formerly occupied by the equatorial plates of the spindles.

17. At the reconstitution of the granddaughter nuclei, young nucleoli

arise in contact with the chromosomes. When the resting condition is established, a crystal body is present in the single nucleolus of each microspore nucleus.

18. Quadripartition of the mother-cell is brought about by furrowing, the furrows appearing to be formed by the ingrowth of wedge-shaped masses from the surrounding thick wall.

19. The microspores are liberated in the loculus by the dissolution of the surrounding material.

20. The tapetal cells remain uninucleate throughout the whole process of pollen development.

21. Sterility may become apparent at any stage in the pollen development following diakinesis.

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I wish here to express my indebtedness and gratitude to Professor Gates for his guidance, encouragement, and kindly criticism throughout the investigation.

#### ADDENDUM.

Since this paper was prepared for publication I have been able to examine a quantity of material collected in March 1925, for which I am indebted to Mr. G. N. Bunyard. The fixatives used were strong Flemming, Merkel, and Hermann. In every case the material revealed features similar to those described above.

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## EXPLANATION OF PLATES X-XII.

Illustrating Miss Jean Latter's paper on the Pollen Development of *Lathyrus odoratus*.

All the figures were drawn with a camera lucida, and have been reproduced without reduction.

With the exception of those mentioned below, all were drawn under a  $\frac{1}{2}$ -inch. imm. Swift N.A. 1·25, with Comp. Oc. 12. Magnification.  $\times 2,400$ .

Figs. 12, 2, 10, 11, 12, 13, were drawn under a  $\frac{1}{2}$ -inch imm. Swift N.A. 1·30 with Comp. Oc. 12. Magnification.  $\times 3,000$ .

Fig. 15 was drawn under a  $\frac{1}{2}$ -inch imm. Swift N.A. 1·25 with Comp. Oc. 8. Magnification.  $\times 1,600$ .

### Abbreviations.

A.A. = Acetic alcohol fixative.

A.B. = Allen's Bouin fixative.

C.A. = Chrome-acetic fixative.

### PLATE X.

Fig. 1. A 'resting' pollen mother-cell. The crystal body is deposited within the central vacuolate region of the nucleolus. The reticulum has a very granular appearance. (A.B.)

Fig. 12. A nucleolus containing a crystal body of triangular shape. The central vacuolate region, within which the crystal body is deposited, is flat-sided. (A.B.)

Fig. 2. A tapetal cell with crystal bodies in the nucleolus.

Fig. 3. Very early prophase. The reticulum contracts from the nuclear membrane. (A.B.)

Fig. 4. The nucleolus is passing to the periphery of the contracted reticulate mass, and assuming an elliptical form. (A.B.)

Fig. 5. The nucleolus is in contact with the nuclear membrane and slightly flattened against it. Very delicate threads are becoming apparent in the reticulum. (A.B.)

Fig. 6. Further flattening of the nucleolus against the nuclear membrane. Considerable increase in the nuclear area is now evident. (A.B.)

Fig. 7. Nucleolar budding. (A.B.)

Fig. 8. Nucleolar budding. The granular reticulum is becoming distinctly thread-like. (A.B.)

Fig. 9. An amoeboid type of nucleolus. (A.B.)

Fig. 10. A very faintly stained nucleolus containing minute dark-staining granules which are probably fragments of the crystal body shown in Fig. 1. The reticulum was too lightly stained to detect the delicate threads. (A.B.)

Fig. 11. Early synizesis. The knot is now composed of an extremely delicate tangled thread and is beginning to move away from the nucleolus across the nuclear cavity. One refractive-looking 'crystal body fragment' remains in the nucleolus and is attached on one side to a strand from the synizetic knot. This preparation was too faintly stained to allow of following the course of this strand across the nucleolus. A small additional nucleolus is present in the nuclear cavity. (Also in Figs. 12 and 13.)

Fig. 12. Similar stage of development to Fig. 11. A loop of delicate thread is in contact with the one remaining 'crystal body fragment'. The nucleolus is lying in a lower focus than the other nuclear contents, probably against the nuclear membrane. (A.B.)

Fig. 13. The synizetic knot is now farther away from the nucleolus in which the peripheral nucleolar body is apparent. A loop of thread from the knot is connected to the nucleolar body. (This series of figures strongly supports the view that the nucleolar body is derived from the crystal body of the resting nucleus.) (A.B.)

Fig. 14. Typical synizesis. No indication of the granular character of the synizetic knot remains. A strand of thread is seen looping over the nucleolus and is connected to a small nucleolar body. (A.B.)

Fig. 15. Three nuclei showing extreme cases of nucleolar flattening against the membrane. (The thread in these nuclei is loosening from the synizetic knot.) (A.B.)

Fig. 16. Loosening of the spireme from synizesis. The preparation is too deeply stained to detect the nucleolar body. Probably the projecting portion of the nucleolus, to which a loop of thread is attached, marks its position. (A.B.)

Fig. 17. The spireme is entirely loosened from the synizetic knot. It is a perfectly continuous thread, becoming beaded with chromatin granules. The attachment of the thread to the nucleolar body is clearly seen. The nucleolar body has increased considerably in size. (The object of this figure being to show the association of the thread and the nucleolar body, the entire volume of spireme is not depicted. This remark also applies to Fig. 18.) (A.B.)

Fig. 18. Same as Fig. 17. The nucleolus is lying against the upper membrane of the nucleus, being in a focus higher than the other nuclear contents. (A.B.)

Fig. 19. A spireme loosening from synizesis in a nucleus in which the synizetic knot remained in a lateral position near the nucleolus. The nucleolus is of an irregular shape. The nucleolar body is not discernible from the remainder, but is probably situated in the projecting nucleolar bud to which the thread is attached. (A.B.)

Fig. 20. Seven persistent loops can be distinguished from a mass of smaller loops and are lettered *a-g*. A split is present in the thread of some of the main loops, but this is not generally observed in nuclei at this stage. The loops appear to radiate from a central nucleolus. On account of this dark-staining nucleolus the continuity of the spireme cannot be seen. No nucleolar body can be detected in the peripheral nucleolus. (A.B.)

Fig. 20a. The seven persistent loops of the previous figure are drawn separately, showing the position of each with regard to the central nucleolus. Loops *c, e, f, g* show the split in the thread.

Fig. 21. A slightly later stage than Fig. 20. The nucleolus completely obscures the course of the thread in contact with it. The seven loops are lettered *a-g*. A small bud projects from the nucleolus. (A.B.)

Fig. 21a. The seven loops of spireme of the previous figure are drawn separately, showing the relative position of each to the nucleolus. No split is present in these loops.

Fig. 22. Typical bronchonema stage. The seven loops radiate from the centre of the nucleus, giving the appearance of a seven-spoked wheel. The arms of certain of the loops are seen intimately twisted round one another, giving opportunity for exchange of segments between homologous chromosomes. The nucleolus begins to round off from the membrane. The nucleolar body is present at the apex of the nucleolus, but is no longer connected to the spireme. That part of the thread which is nearest the nucleolar body is very irregular in outline, indicating that separation from the nucleolar body may recently have occurred. (A.B.)

Fig. 23. Bronchonema stage. An association between the spireme and nucleolar body is still evident. The arms of certain loops are in intimate contact. (A.B.)

#### PLATE XI.

Fig. 24. A slightly later stage than Fig. 23. Condensation of the spireme is becoming more pronounced. The two arms of each loop are free from one another. The seven loops are lettered *a-g*. The large nucleolus is rounded off from the membrane. A small nucleolus is situated at the centre of the nucleus. (A.B.)

Fig. 25. Segmentation of the spireme into bivalent chromosomes. Loop *g* has already assumed the 'figure of eight' form. The nucleolus shows a tendency to 'bud'. Two small nucleoli are present in addition to the large peripheral one. (A.B.)

Fig. 26. Further segmentation of the spireme, showing the connecting strands between the bivalents, only five of which are visible. The chromosomes are very irregular in outline and have a vacuolate appearance. (A.B.)

Fig. 27. Early diakinesis, showing the variability in form of the seven bivalents. The nucleolus is very vacuolate. (A.B.)

Fig. 28. Slightly later than Fig. 27. Condensation of the seven bivalents is not yet completed. Four nucleoli are present. (C.A.)

Fig. 29. Typical diakinesis. The bivalents lie peripherally in the nuclear cavity. No nucleoli could be detected in this nucleus nor in the adjacent sections of the same cell. Probably small nucleoli are lying in direct line with certain of the chromosomes. (C.A.)

Fig. 30. Late diakinesis. The nuclear membrane is much thickened and the nuclear contents just within it appear somewhat 'cloudy'. This 'cloudiness' indicates approaching spindle formation. Four vacuolate nucleoli are present and the seven bivalent chromosomes are becoming massed together. (A.A.)

Fig. 31. A definite spindle sheath is formed round the seven bivalents and two nucleoli. One conical projection of fibres extends into the cytoplasm. The pale-staining character of the nucleoli could not be shown on account of the underlying chromosomes. (A.A.)

Fig. 32. Tripolar spindle. The bivalent character of the chromosomes is obscured. Two nucleolar fragments are seen in the cytoplasm. (C.A.)

Fig. 33. Formation of bipolar spindle. Nucleolar fragments are present in the cytoplasm. (C.A.)

Fig. 34. Early metaphase in which the chromosomes are scattered and their bivalency very evident. This figure indicates differences in size of the chromosomes, but this is not generally observed. (C.A.)

Fig. 35. Typical heterotypic metaphase. Only six bivalents are visible on the equatorial plate. (C.A.)

Fig. 36. Early heterotypic anaphase. The split in the univalent chromosomes is apparent, causing them to appear as V's as they draw apart from one another. (A.B.)

Fig. 37. Slightly later heterotypic anaphase. Seven univalents are visible in the upper row, and six in the lower row of chromosomes. (C.A.)

Fig. 38. Heterotypic anaphase. A rather foreshortened view of the spindle, only one pole of which is discernible. Six univalents are present in the upper and seven in the lower group of chromosomes. (A.B.)

Fig. 39. Heterotypic telophase. Certain chromosomes in the lower group reveal the homo-typic split. The former position of the spindle is marked by striations in the cytoplasm. (A.B.)

Fig. 40. Interkinesis. Seven chromosomes are visible in each group. The two halves of each univalent chromosome frequently lie across one another. The chromosomes in the lower plane are

shaded faintly; their staining character is similar to that of those in the upper group. (Also in Fig. 41.) (A.B.)

Fig. 41. Interkinesis, showing the elongation of the chromosomes whose dual nature is evident. Only five chromosomes are seen in the further group. Slight indications of a nuclear membrane are present. (A.B.)

Fig. 42. Later interkinesis. Only one daughter nucleus is shown. Anastomosing strands are seen between the seven univalent chromosomes, and dark-staining extruded masses are in contact with them. A delicate nuclear membrane is formed. (A.B.)

Fig. 43. Reorganization of chromosomes for the homotypic division. The homotypic split is completely obscured. Seven chromosomes are present in each group. (A.B.)

## PLATE XII.

Figs. 44 and 45. Homotypic metaphase. (A.B.)

Fig. 46. Homotypic anaphase. Only one group of chromosomes is shown in connexion with the lowermost spindle. A thick wall of homogeneous appearance surrounds the cytoplasm. (A.B.)

Fig. 47. Homotypic telophase. The chromosomes are very compact and deep-staining. The spindle fibres are represented by striations in the cytoplasm. Note the angular outline of the thick wall, corresponding to the outline of the pollen mother-cell which has not become rounded off. (A.B.)

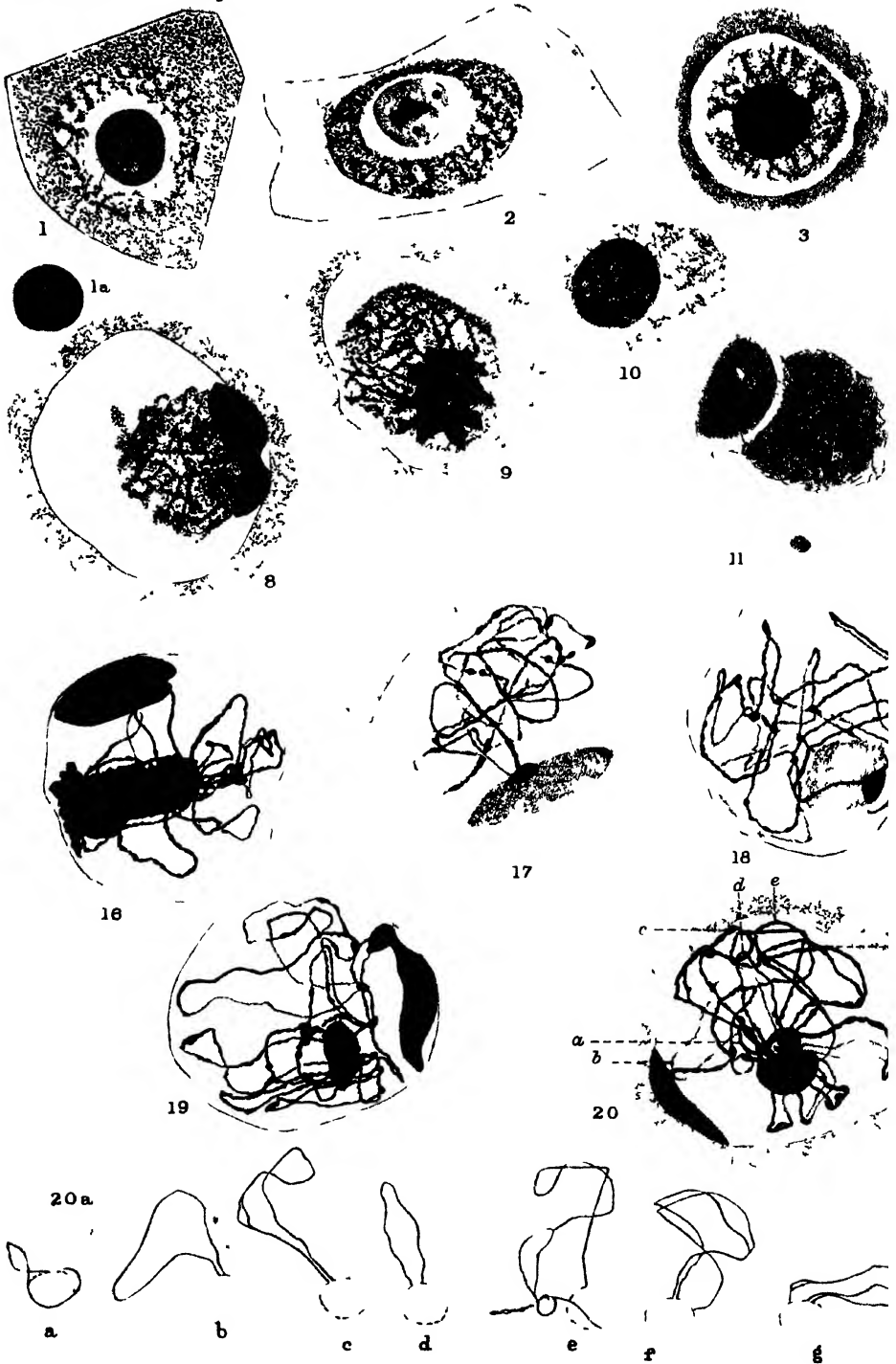
Fig. 48. Evanescent cell-plates are seen across the equatorial plates of the 'spindle striations'. This preparation is very deeply stained, the chromosomes being indistinguishable from one another. Contraction of the cytoplasm from the thick wall has taken place. (A.B.)

Figs. 49 and 50. Reconstitution of the granddaughter nuclei. The chromosomes are anastomosing with one another and losing their deep-staining character. Young nucleoli appear and are usually first seen in contact with the chromosomes. The thick wall has increased in size. (A.B.)

Fig. 51. Quadripartition of the mother-cell protoplast by furrowing. A membrane can be seen surrounding the cytoplasm where contraction from the thick wall has occurred. The nuclei appear to be becoming abortive. (A.B.)

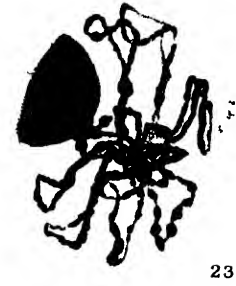
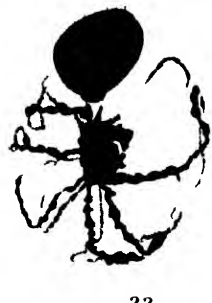
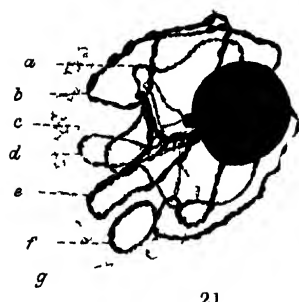
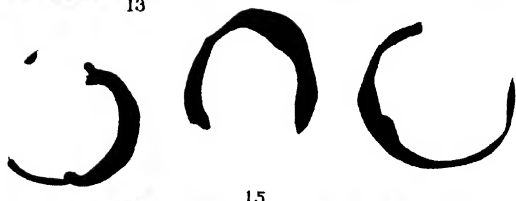
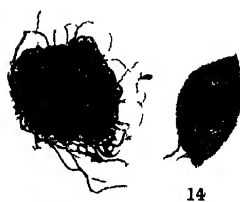
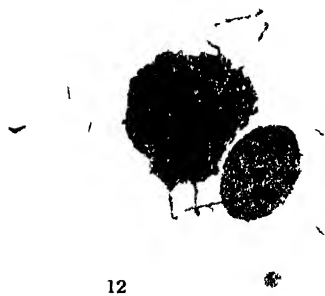
Fig. 52. Pollen tetrad, only three members of which are drawn. The microspores are completely separated from one another by ingrowths of the thickened wall. Enormous enlargement of the microspores has occurred. The cytoplasm of each is surrounded by a membrane. The resting condition is established in the nuclei. (A.B.)

Fig. 53. Dissolution of the thick wall surrounding the tetrad. A wall surrounds each microspore, and shows indications of pore formation. A crystal body is present in the nucleolus of each nucleus. The contents of all the members of the tetrad are similar, only one being depicted in detail. (A.B.)



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LATTER — POLLEN DEVELOPMENT.



b

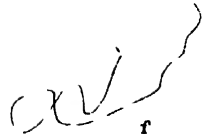
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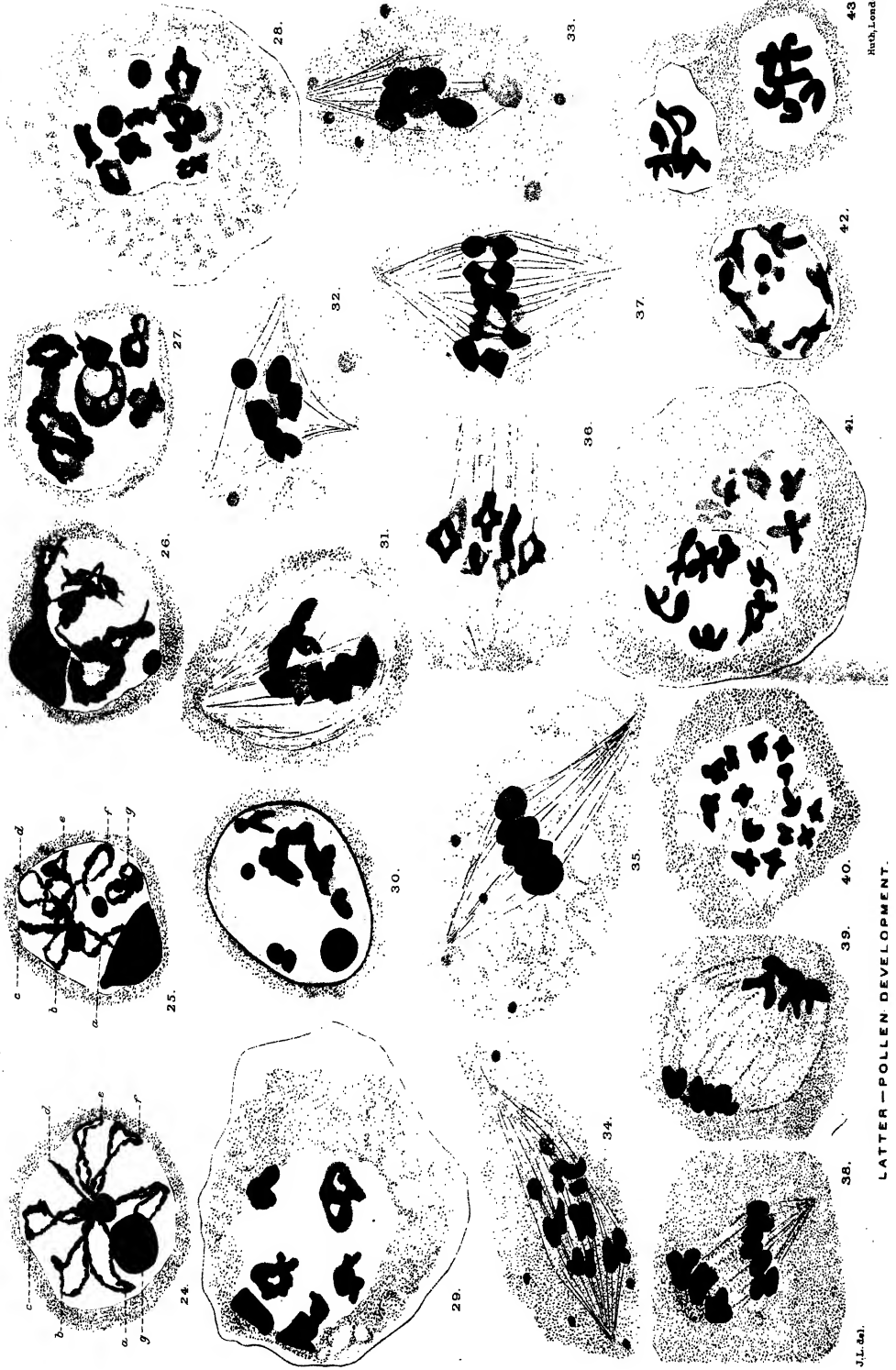
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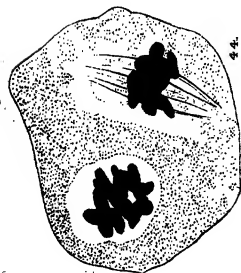


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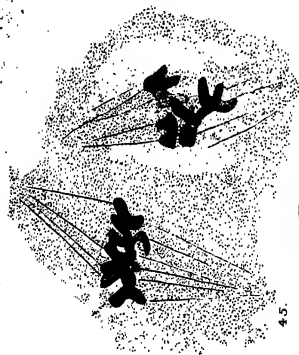


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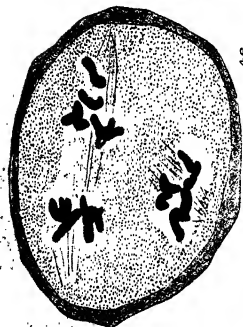




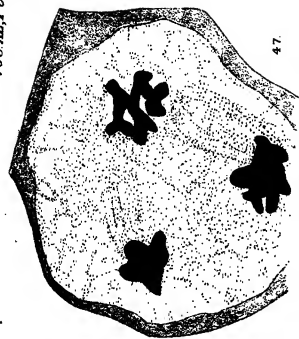
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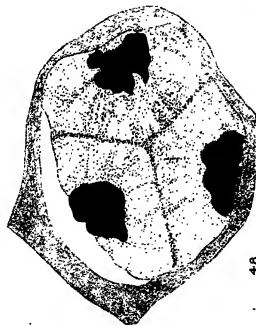
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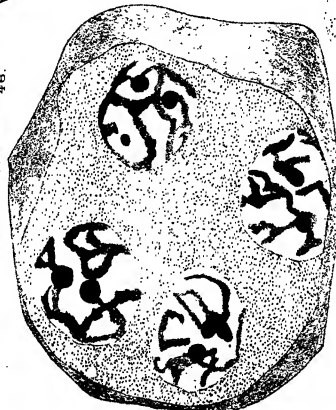
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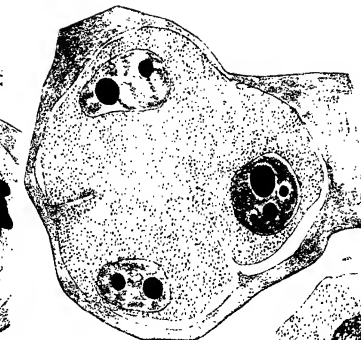
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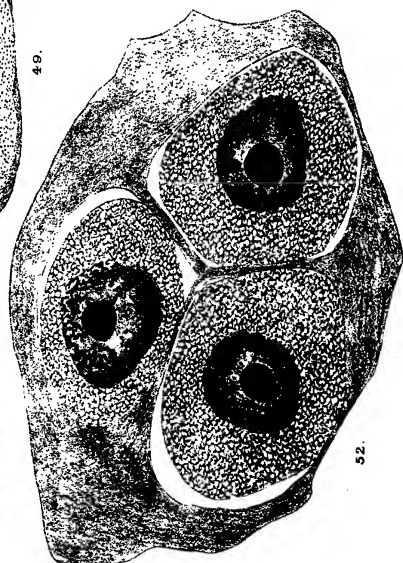
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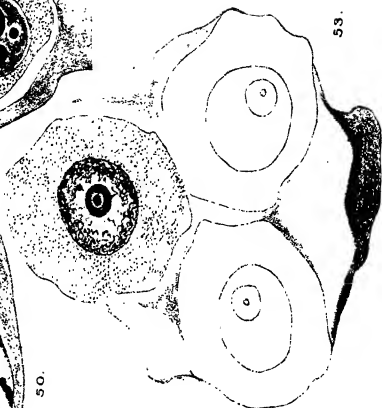
49.



50.



52.



53.

51.

LATTER — POLLEN DEVELOPMENT.





# The Response of Certain Photoperiodic Plants to Differing Temperature and Humidity Conditions.

BY

BASIL E. GILBERT.

With two Figures in the Text.

## INTRODUCTION.

AMONG the problems of interest to botanists in general, and physiologists in particular, those connected with the periodic flowering of different plant species are of much importance. Garner and Allard (1) have shown that with certain species, the relative day length may be the dominant causal influence in the termination of vegetative activity. They, moreover, have not lost sight of the importance of other environmental factors, and have drawn attention particularly to the effect of temperature in connexion with the response to relative day length with Soy Beans (2). This paper draws attention to the reactions of certain plants known to respond to relative day length, when grown under two controlled sets of temperature and humidity conditions.

## EXPERIMENTAL.

Plants of different varieties of Soy Beans, *Cosmos*, *Salvia*, Cotton, and Buckwheat were grown from seed in eight-inch unglazed porcelain pots. The soil was a rich sandy garden loam containing sufficient organic matter to preclude any possibility of fertilizer limitations. The plants were grown under two sets of conditions in greenhouses where both temperature and humidity were mechanically controlled. The temperatures of the two houses were recorded on daily record sheets by electrical recording thermographs. Figs. 1 and 2 indicate the temperature fluctuations over the entire growth period. The points on the curves represent daily averages obtained by planimetric calculation from the record sheets. Owing to mechanical



difficulties it was found impossible to obtain the same humidities in both houses and so the humidifier was adjusted for the cool condition. The humidities of the two houses were controlled by the circulation of air, and thus were very constant. The relative humidity of the cool house was

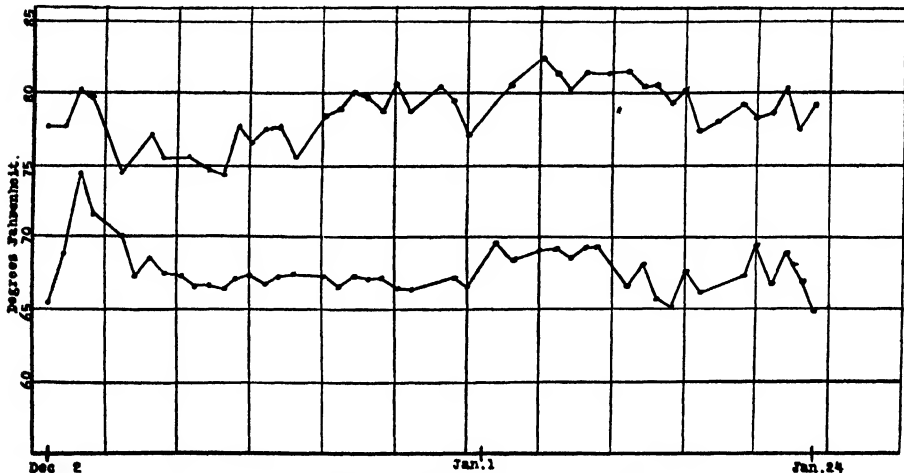


FIG. 1. Graph showing average daily fluctuations of temperature.

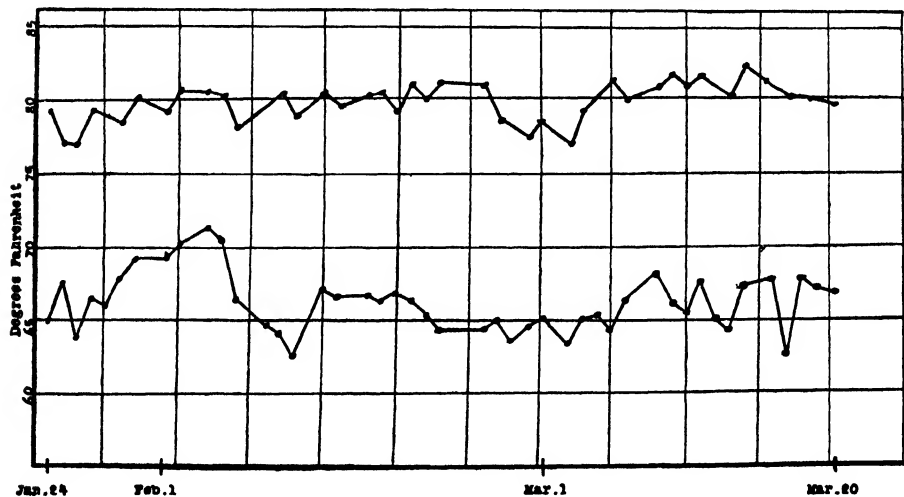


FIG. 2. Graph showing average daily fluctuations of temperature.

maintained at 85 per cent., while that of the warm house remained at 50 per cent. The entire growth period covered a portion of the year when the relative day length was short. In every case comparisons were made with plants grown under the two sets of conditions. Thus while relative day length was constant, temperature and humidity were the variables.

OBSERVATIONS.

Weekly observations were made and a record kept of the dates of planting, appearance of first buds, and when 50 per cent. of each species bore flowers which were in open bloom. Measurements were also made weekly of individual plants, and the average results serve to indicate the rate of growth. Table I contains the record of the planting and flowering dates, while Table II gives the observations of the growth measurements.

TABLE I.

*Contrasting the time of blooming of plants grown under low temperature, high humidity conditions, with those grown under high temperature, low humidity conditions.*

Species.	Date of Planting.	Appearance of First Buds.		Date of 50 % Bloom.	
		warm	cool	warm	cool
SOY BEANS					
Biloxi	Dec. 1	Jan. 2	—	—	—
Mandarin	Dec. 1	Jan. 2	—	—	—
Pekin	Dec. 1	Jan. 2	—	—	—
Tokyo	Dec. 1	Jan. 2	—	—	—
SALVIA					
Bonfire	Nov. 18	Jan. 20	Feb. 6	Feb. 13	Feb. 13
COSMOS					
Lady Lennox	Jan. 19	Mar. 6	Feb. 20	—	Mar. 13
COTTON					
Improved King	Nov. 17	Feb. 6	—	Feb. 27	—
BUCKWHEAT					
Japanese	Dec. 2	Dec. 24	Dec. 24	Dec. 28	Dec. 26

TABLE II.

*Showing weekly average measurements of growth expressed in inches from the surface of the soil to the apical stem region.*

	SOY BEANS.								SALVIA.		COSMOS.		COTTON.		BUCK- WHEAT.	
	Biloxi.		Mandarin.		Pekin.		Tokyo.		Bonfire.		Lady Lennox.		Improved King.		Japanese.	
	warm	cool	warm	cool	warm	cool	warm	cool	warm	cool	warm	cool	warm	cool	warm	cool
First week	8.1	5.3	5.7	3.5	5.5	3.2	6.2	4.1	—	—	—	—	—	—	5.4	4.9
Second week	9.7	6.6	6.7	4.2	6.2	3.7	7.4	5.0	—	—	—	—	—	—	6.2	5.8
Third week	11.5	6.8	7.7	4.4	7.3	4.0	9.0	5.7	—	—	4.9	3.2	2.8	2.0	7.4	7.4
Fourth week	12.7	7.2	8.3	4.8	7.9	4.2	10.9	6.4	—	—	5.6	4.3	3.2	2.0	—	—
Fifth week	12.8	8.2	8.4	5.2	10.9	4.6	10.9	7.2	—	—	7.6	6.3	4.2	2.2	—	—
Sixth week	—	—	—	—	—	—	—	—	2.3	2.0	10.1	8.2	4.6	2.5	—	—
Seventh week	—	—	—	—	—	—	—	—	2.9	2.7	13.0	15.9	5.1	2.6	—	—
Eighth week	—	—	—	—	—	—	—	—	4.3	3.6	15.7	17.4	6.2	2.9	—	—
Ninth week	—	—	—	—	—	—	—	—	5.4	5.1	—	—	7.3	2.9	—	—
Tenth week	—	—	—	—	—	—	—	—	6.3	6.1	—	—	8.5	3.1	—	—
Eleventh week	—	—	—	—	—	—	—	—	7.2	7.5	—	—	9.5	3.2	—	—
Twelfth week	—	—	—	—	—	—	—	—	8.5	9.0	—	—	11.7	3.2	—	—
Thirteenth week	—	—	—	—	—	—	—	—	9.3	10.8	—	—	11.8	3.5	—	—

In addition to the observations recorded in Table I other data of interest were noted with the *Cosmos* plants. Accompanying the marked lengthening of vegetative activity which took place under higher temperature and lower humidity conditions were certain very marked gross characteristics of the growth habit. While the lower temperature plants were strongly vegetative and had abundant strengthening tissue in their stems, the higher temperature plants were greatly etiolated in habit. The latter had leaves which were smaller and more divided in character, the stems were twisted and had little strengthening tissue. What few flower heads resulted were small and poorly formed. So definite were these gross characteristics in an initial experiment that it was deemed wise to attempt their duplication. The experiment reported in Table I showed that the results were confirmatory in every respect.

#### DISCUSSION.

**Soy Beans.**—The observations of Garner and Allard of the modification of the photoperiodic response induced by temperature with Soy Beans are substantiated in the main by the above results. The flowering date was definitely retarded with all varieties by the lower temperature conditions. One experiment only was attempted with Soy Beans and was discontinued on January 2, at which time no indications of buds were noted with the plants in the cool house, while all varieties were in bud and bloom in the warmer conditions. With these two sets of conditions, however, no such varietal differences in length of vegetative activity as those reported by Garner and Allard (3) to be normal with plantings in the latitude of Washington were observed. This is significant when it is remembered that the variable environmental factors were temperature and humidity, and that the relative day length remained constant for both sets of plants.

**Salvia.**—Under the described growth conditions no appreciable difference in the length of vegetative activity was noted. Both plants grew with profuse vegetation and flowered well. It is suggested that conditions giving a wider range of difference would have brought a response, and that the better conditions for growth are found over a wider range of temperature and humidity than with the other plants used in the experiment.

**Cosmos.**—This plant responded best to the lower temperature and higher humidity conditions, both in its growth habit and the shortening of its vegetative activity. In comparison with other plants of the experiment where the positive response was obtained in the warm house, *Cosmos* gave what might be termed a negative response. This leads to the consideration of the environmental conditions which may be considered to be optimum for a positive response. In the case of this plant these conditions obviously were found in the lower temperature house, and the set of condi-

tions obtaining in the higher temperature house may be considered to have been beyond the optimum range, even as the lower temperature and higher humidity conditions were beyond the optimum range for Soy Beans.

Cotton.—Although the writer has no knowledge of any work to show that Cotton exhibits a positive reaction to relative day length, this plant was included as likely to be very sensitive to temperature variations. Plants germinated November 17 under the warm conditions were moved to the cool house on December 9. At that time they measured an average height of 2.0 in. Although remaining perfectly healthy plants, they showed no sign of flowering on February 27, and at that time measured 3.2 in. The plants which were left in the warm conditions grew with profuse vegetation, had many flowers, and set bolls of normal size. So marked was the reaction to these two varying sets of conditions that Cotton should furnish an excellent plant for temperature studies.

Buckwheat.—Under both sets of conditions excellent vegetation and profuse flowering were obtained. No appreciable difference in the time of flowering was noted, and hence Buckwheat may be classed with *Salvia* with respect to the breadth of the temperature and humidity range in which no modification of the relative day length is evidenced.

If the factors other than relative day-length environment in which the plant has its growth be considered, temperature as affecting metabolic processes should be expected to exert an influence on the determining of the time of flower primordia formation. Next in order, humidity as accelerating or retarding transpiration should receive consideration. Hence the study of the response of photoperiodic plants carried on under controlled conditions of temperature and humidity and of known relative day length might be expected to throw light on the conditions obtaining at and before primordia formation, and also to give indications as to the importance of relative day length as contrasted with these other factors. In this experiment the modification of the response obtained proved so great that it seems illogical to ascribe more of the role of the causal agency to relative day length than to temperature or humidity.

#### SUMMARY.

It is suggested that the phenomena of response to relative day length may be materially influenced by the particular temperature and humidity conditions to which the plant may be subjected during its growth period. In this experiment certain plants known to react to relative day length were subjected to two sets of temperature and humidity conditions. Marked results were obtained in the modification of the length of the vegetative activity.

Dependent upon the species one or the other set of conditions proved best for the modification of the response to relative day length.

320 *Gilbert.—The Response of Certain Photoperiodic Plants.*

1. Soy Beans and Cotton exhibited definite reactions to the higher temperature and lower humidity conditions. Definite retardation of flowering was noted with the lower temperature and higher humidity conditions.

2. *Cosmos* also was definite in its reaction, but flowered much earlier and more normally under the lower temperature and higher humidity conditions.

3. *Salvia* and Buckwheat exhibited no reaction to temperature and humidity conditions such as used in this experiment.

The writer is deeply grateful to Dr. William Crocker of the Boyce Thompson Institute for Plant Research for his encouragement and kindly criticism, and also for placing at his disposal the controlled greenhouses in which the plants were grown.

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2. ————— : Further Studies in Photoperiodism, the Response of the Plant to Relative Length of Day and Night. Journ. Agr. Res., xxiii, 871-920, 1923.
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# The 'Leaf' of *Nitella opaca*, Ag., and Adventitious Branch Development from it.

BY

KATHLEEN M. DREW, M.Sc.

With fifty-five Figures in the Text.

## I. INTRODUCTION.

ALTHOUGH the Characeae include six well-defined genera, numerous investigations have not only made the anatomical construction familiar but have shown that this is essentially the same throughout the group. Each shoot increases by apical growth. The apical cell cuts off segment cells, each of which divides into a lower internodal and upper nodal cell, a rule which holds throughout most cell divisions in this family. The internodal cell does not divide again, but elongates very considerably, often reaching a length of several centimetres. The upper nodal cell divides and a whorl of short branches ultimately results, giving the characteristic appearance to the plant. The structure of these branches is often not only characteristic for the genus but is a distinguishing feature of the species, especially in *Nitella*. Although a more suitable name would be branches of limited growth, they have been called 'leaves' by analogy with those organs of higher plants, to which the Characeae were once supposed to be more closely related. Although it is proposed to retain the term 'leaf' for convenience in the description which follows, it is used in this special sense and means nothing more than a branch of limited growth.

In addition to the whorl of 'leaves' other branches are to be found at every node. They repeat the structure of the main axis and are known as the axillary or lateral branches. In most cases only one develops at each node and then it is invariably found in the axil of the oldest 'leaf', but in *Nitella syncarpa*, Chevall, as Giesenhagen (2) has shown, two develop, one from the basal node of each of the oldest two 'leaves'.

It was Pringsheim (10) (1862) who first found that under certain con-

ditions nodes of *Chara fragilis*, Desvaux, produced numerous branches in addition to the one normal lateral branch. In nature, old nodes which had survived wintery or other unfavourable conditions were found with such branches, and later Pringsheim found that their formation and development could be experimentally induced by isolating the nodes of the main axis or branches. Although species of *Chara* produce these branches most readily, other investigators (2, 3, 6, 11) have found that species of *Nitella* and *Tolypella* can be stimulated to form accessory and adventitious branches, and attention has been focused not only on their exact place of origin but also on the conditions under which they develop. From the results of the work of these investigators, it has become clear that a distinction should be made between branches which develop under natural or experimental conditions from dormant apices, always found in a definite position and which may be called accessory branches, and those which develop in no special place and may therefore be spoken of as adventitious. Kuczewski (6) showed that at the base of every lateral branch of *Chara delicatula*, f. *bulbillifera*, A. Braun, there are always two dormant apices, which may be termed accessory branches, and which never develop unless the lateral branch is damaged or removed. Similarly, secondary accessory branches are often formed at the base of the primary ones and they always bear exactly the same relation to the primary as the latter do to the lateral branch.

Whereas the accessory branches are always of the radial type, that is like the main axis, the adventitious branches are more often pro-embryonic, that is like the pro-embryo, which results from the germination of the spore. They were named Zweigvorkeime by Pringsheim. The first nodes of the radial branches, whether accessory or adventitious, are very often of a simpler type than usual. This is especially evident in the Charae where the first internode may not be corticated, the cortical filaments being either entirely absent or hanging free from the node. On account of this character Pringsheim called them 'nacktfüssige' branches. In the Nitelleae the simplification is seen in the structure of the 'leaves' only.

Experiments have been carried out with *Chara vulgaris*, L. (*C. foetida*, Braun), and they have shown that when nodes are isolated and the lateral branch cut from each such node, accessory and adventitious branches are formed, as Pringsheim (10) described for *C. fragilis*. The first to develop are the primary accessory branches, which occur in this species as in *C. delicatula* described by Kuczewski (6), and then, in the axils of the youngest 'leaves' only, adventitious branches, which are almost without exception pro-embryonic. There are, therefore, two centres of development at the node—one, that of radial branches from the base of the lateral branch, and the other that of pro-embryonic adventitious branches on the opposite side of the node. If an isolated node is cut in halves longitudinally, the accessory and adventitious branches still develop. A very extensive

development of adventitious branches results if the node is wounded by removing the lateral branch and the oldest 'leaf', with their basal nodes. Similar results have been obtained with *Chara hispida*, L., which produces very large numbers of accessory and adventitious branches, as many as forty five having been counted at one node. In addition, adventitious branches, both radial and pro-embryonic, have been stimulated to develop from the stem-node of pro-embryos by isolation of the node and removal of the existing lateral branch.

Since these experiments add but little to the results already described by other investigators, they will not be described further, but some experiments with *Nitella opaca*, Ag., have shown that this species is exceptional in that it has the power to develop adventitious branches not only from the basal but also from the upper node of the 'leaf', unlike *Chara fragilis* and other Characeae. It is especially the structure of the 'leaf' and the development of adventitious branches from its upper node which are to be described in this paper.

*Nitella opaca* is the commonest British species and is nearly allied to *N. syncarpa*, Chevall. At each node there are most commonly eight 'leaves' which are quite simply constructed, having only one node above the basal node. Only six of these 'leaves', however, really belong to the primary whorl. Like *N. syncarpa*, this species forms two lateral branches at each node, one from the basal node of each of the oldest two 'leaves'. A study of their development shows that, as Giesenhagen (2) has described for *N. syncarpa*, each branch is lateral to, and not in, the axil of the 'leaf' from the basal node of which it develops. There is a further complication due to the formation of a 'leaf' at the base of each of these branches, so that the 'leaf' in the axil of which the axillary branch appears to stand is really a secondary 'leaf' and not one of the original whorl.

In summer, when the plants are fertile, as many as eight lateral branches may be found at a single node, and in such cases there is a corresponding increase in the total number of 'leaves' in the whorl.

## II. THE STRUCTURE OF THE 'LEAF' OF *NITELLA OPACA*, AG.

In contrast to the other genera of the Characeae the structure of the 'leaf' varies considerably in the genus *Nitella*. In one group including *N. opaca*, *N. syncarpa*, and *N. flexilis*, the 'leaf' has only one node, in addition to the basal node. Although more initials are formed, 'leaflets' are only found on the adaxial side of this upper node. There are usually two, and each is simply an elongated cell like the terminal segment of the 'leaf', which is the modified apical cell.

The other extreme is represented by *N. mucronata*, *N. gracilis*, &c. Each 'leaf' in these species has several nodes, from each of which 'leaflets'

branch out. These in their turn may have two or three nodes bearing 'leaflets', the older ones of which may again be noded. It is evident that such a structure is very branch-like but differs from the main axis in that the apical growth is limited, for after a certain number of divisions (usually constant for the species) the apical cell ceases its activities and elongates considerably, becoming the terminal segment of the 'leaf'. In between these extremes are forms, e.g. *N. subtilissima* and *N. congesta*, which in the fertile condition have 'leaves' with many nodes, whereas the 'leaves' of sterile plants have only one. The 'leaves' of *N. cernua* are interesting, since each 'leaf' has one node only, but the 'leaflets' form a closed whorl unlike *N. opaca*. The 'leaf' in all species has a basal node just as a lateral branch, and in the case of the more complex 'leaves' of the *N. mucronata* type each 'leaflet' has a basal node as well. In the Charae, it is quite obvious, from the sequence of the divisions, that the basal internode and basal node of the 'leaf' represent the product of the first segment cell and only differ from other homologous cells in that the internodal cell does not elongate and that the divisions in the nodal cell are modified. In *Nitella*, on the other hand, the first segment cell does not divide to give an internodal cell, but itself becomes the basal nodal cell. Excepting *N. hyalina* and *N. congesta*, no branches or 'leaflets' develop under normal conditions from the basal nodes of the 'leaves' of *Nitella* except the first one, or in some cases two, of each whorl, which give rise to the lateral branches.

The so-called 'leaves' differ, therefore, from the main axis by their limited growth and by the simplification in the structure of the lateral appendages at the nodes. In the *Nitella opaca* type these differences are most marked, but in some other species, e.g. *N. mucronata*, much less evident, the 'leaf' being very branch-like.

The structure and development of the 'leaf' of *N. opaca* has been followed in detail. In most of the Characeae each peripheral cell of the node of the main axis develops into one 'leaf', but in *N. opaca*, as has already been mentioned, the oldest two of the six peripheral cells are considerably larger than the others (Fig. 1,  $a_1$  and  $a_2$ ), and unlike the other four ultimately give rise to two 'leaves' as well as a branch. The early stages in the development of all the peripheral cells are the same however. The cell swells and projects beyond the surface of the neighbouring internodal cells (Fig. 3,  $a_2$ ) and then cuts off an inner cell  $s_1$  (Fig. 2), i.e. the first segment cell.

Although the apical cell of the 'leaf' may be quite like the corresponding cell of a branch at first, after cutting off a second segment cell  $s_2$  (Figs. 3 and 4,  $l_1$  and  $l_2$ ) it quickly loses its hemispherical shape, becoming elongated (cf. leaves 1 and 2 of Fig. 4). Like the nucleus of internodal cells the nucleus of the apical cell of the 'leaf' soon fragments and no more cell divisions take place.

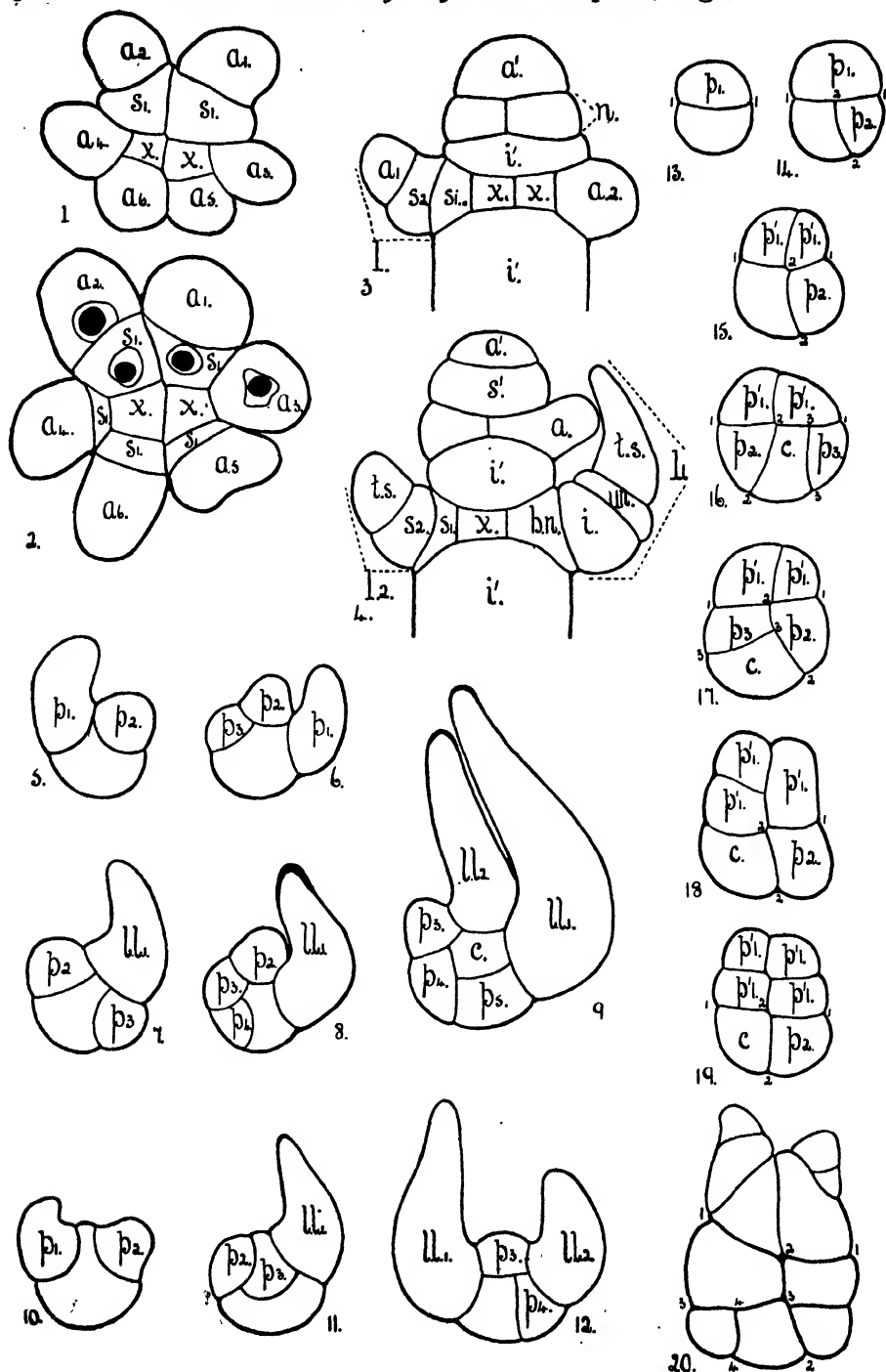
Of the two segment cells cut off by the apical cell, the first never divides, but itself forms the basal node (*b.n.*) of the 'leaf' (Fig. 4, *l.*<sub>1</sub>); but the second segment cell divides into an upper nodal (*u.n.*) and lower internodal (*i*) cell (Fig. 4, *l.*<sub>1</sub>). It is from this nodal cell that the 'leaflets' develop. Occasionally, the second segment cell does not divide, and so a simple type of 'leaf', consisting of two elongated green cells, above a basal node, results. There is no relation between the complexity of the 'leaf' and its position at the node except that the two so-called secondary 'leaves' which develop in relation to the lateral branches are often of the simpler type unless the plant is bearing oogonia and antheridia.

The further development of the two nodes of the 'leaf' has been followed by treating apical buds with Eau de Javelle, which not only renders the object transparent but allows of the separation of the 'leaf' from its basal node, so that it can be seen in surface view and also the separation of the upper node from the rest of the 'leaf'.

Although the divisions which take place in both the basal and upper node of the 'leaf' follow the same rule, yet the mature nodes appear very different. In both nodal cells, peripheral cells are cut off, leaving a central cell. It is evident, at once, that the difference between this method of division and that in the nodal cell of the main shoot is that no halving wall is formed before the peripheral cells are cut off.

In the upper node the peripheral cells form a complete ring and therefore surround the central cell (Fig. 9), but in the basal node only two peripheral cells may be formed and a complete ring never appears to be formed, and so the central cell has some free surface (Figs. 18, 19, *c.*).

In the basal nodal cell of each of the oldest two 'leaves' of each whorl the first division is parallel to the main axis of the shoot. The oldest peripheral cell, therefore, occupies a lateral position and develops into the lateral branch, which gives rise to a secondary 'leaf' from its base. The first division in the basal nodal cell of all other 'leaves' is at right angles to the main axis of the shoot (Fig. 13, *1-1*) on which the leaf is borne, and cuts the cell into two almost equal portions. The upper half represents the first peripheral cell (*p*<sub>1</sub>) and in the lower half a second peripheral cell is cut off (*p*<sub>2</sub>). This may be the last peripheral cell to be formed (Figs. 18, 19) or a third may sometimes be cut off, on the opposite side of the node. The wall which cuts off the third cell may intersect the wall of the first (Fig. 16, *3-3*) or the second (Fig. 17, *3-3*) peripheral cell. Before the third peripheral cell has been cut off, the first has often divided into two daughter cells (*p'*<sub>1</sub>) by a wall which invariably forms at right angles to the first wall (*1-1*). After this there are numerous divisions which are very often irregular, but Figs. 18 and 19 show cases where the daughter cells have again divided into two equal halves. The divisions in the other peripheral cells and the central cell (which may also divide) are always irregular. In the mature node,



FIGS. 1-20. Figs. 1 and 2. Young nodes of the main axis showing the ring of peripheral cells (a) surrounding the central cells (x) and the first division in the peripheral cells to give the first segment

therefore, it is often difficult to distinguish the primary divisions. This development of the basal node agrees with Giesenhagen's (2) description for other species of *Nitella*.

The divisions in the upper node of the 'leaf' result in a ring of five peripheral cells (occasionally four or six) surrounding one central cell (Fig. 9), which may divide later. The first peripheral cell to be formed occupies a somewhat lateral position with respect to the axis on which that 'leaf' is borne (Fig. 5,  $p_1$ ). The second peripheral cell ( $p_2$ ) is usually formed on the adaxial side of the first (Fig. 5), and the third against the second (Fig. 6), and the fourth the third (Fig. 8), and so on until the circle is completed (Fig. 9). Thus, whereas in the nodes of the main shoot and branches the youngest peripheral cell is opposite the oldest, this is only exceptionally the case in the node of the 'leaf' (Fig. 7, where  $p_1$  is between  $p_2$  and  $p_3$ ). In some cases the wall cutting off the second peripheral cell does not join the first (Fig. 10) and so the central cell is left with free surface between the first two peripheral cells. In such a case a wall forms, cutting off this region so that the third peripheral cell ( $p_3$ ) comes to lie between the first and third (Fig. 11). When this happens the fourth wall may bridge across from the periphery of the nodal cell to the third wall and not the second (Fig. 12). After all the peripheral cells have been cut off, the central cell may divide again either once (Fig. 24) or twice (Fig. 23). In that it is capable of division it resembles the central cells of the nodes of the main axis.

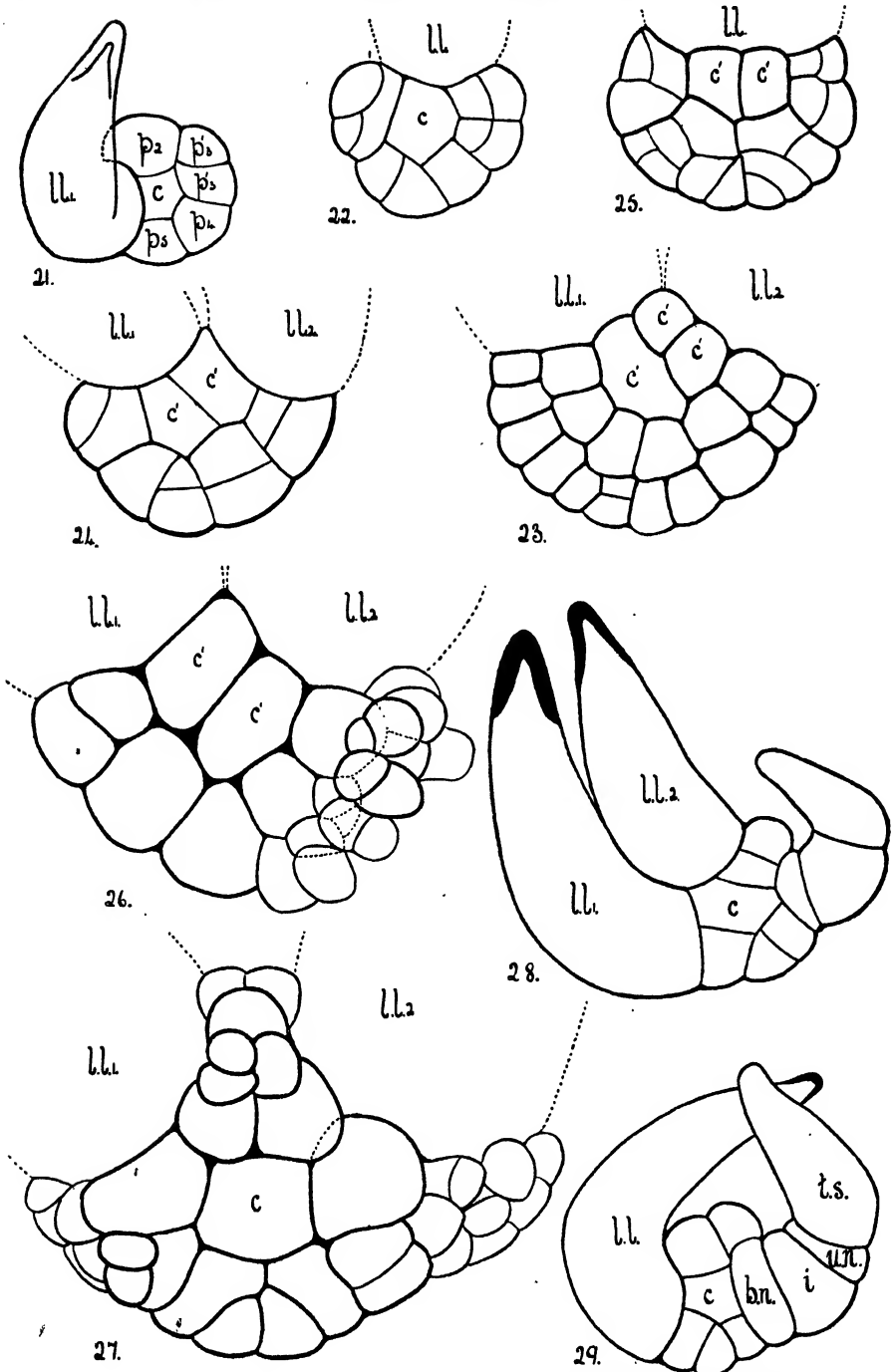
*Nitella opaca* agrees with the other Characeae, which have been investigated in the absence of a halving wall in the upper node of the 'leaf'. Unlike *Nitellopsis obtusa*, J. Groves, described by Giesenhagen (3), and the younger nodes of *Nitella hyalina*, (D.C.) Ag., described by Ernst (1), the ring of peripheral cells appears always to be completed in *N. opaca*. Also only exceptionally do the divisions take place alternately on opposite sides of the node as is described for *Nitellopsis obtusa* and for *Chara contraria*, A. Braun, and *Chara dissoluta*, A. Braun, by Sluiter (12).

The further development of each of the five peripheral cells of the upper node of the 'leaf' of *N. opaca* varies considerably, but within more or less definite limits. In the sterile condition, most commonly the first and second, but quite often the first only or the first three, peripheral cells

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cell ( $s_1$ ) of the 'leaf'.  $\times 280$ . Figs 3 and 4. Longitudinal section of the apex of the shoot, showing stages in the development of the 'leaf' ( $l$ ).  $\times 280$ . Figs 5-12. Early stages in the development of the upper node of the 'leaf' and the 'leaflets'.  $\times 280$ . Figs. 13-20. Early stages in the development of the basal node of the 'leaf'. Figs. 13-17 and 19.  $\times 280$ . Figs. 18-20.  $\times 160$ .

$a$ . = peripheral cell of node of main axis, i. e. apical cell of 'leaf';  $a'$  = apical cell of main axis;  $b.n.$  = basal node of 'leaf';  $c$ . = central cell of nodes of 'leaf';  $i.$  = internodal cell of 'leaf';  $l$ . = 'leaf';  $l.l.$  = 'leaflet';  $p$ . = peripheral cell of nodes of 'leaf';  $p'$ . = daughter cell of peripheral cell;  $s$ . = segment cell of 'leaf';  $s'$ . = segment cell of main axis;  $t.s.$  = terminal segment of 'leaf'; i. e. modified apical cell;  $u.n.$  = upper node of 'leaf';  $x$ . = central cell of node of main axis.



FIGS. 21-9. Figs. 21-7. Surface view of upper node of the 'leaf', showing the divisions in the central and peripheral cells. Fig. 21. The third peripheral cell only has divided, and in Fig. 22 all



develop into 'leaflets'. There are no divisions, the cell simply protruding, at first keeping its rounded shape but later becoming more elongated and the tip becoming more mucronate (Figs. 5-9). The resulting structures are in every way similar to the terminal segment of the 'leaf', i. e. the modified apical cell.

In rare cases a 'leaflet' may be replaced by a more complex structure, essentially like a 'leaf'. Fig. 55 shows the upper node of one of the oldest two 'leaves' of a whorl. From the node two lateral structures have developed. One of these is of the usual type, i. e. unicellular, but the other is exactly like a 'leaf' with a node from which a young unicellular 'leaflet' is growing. Similar but younger stages are seen in Figs. 28 and 29, where the upper node of each 'leaf' is shown in surface view. In Fig. 29, as in Fig. 55, two lateral structures are forming, one unicellular and the other consisting of four cells. This structure is exactly like a young 'leaf', before the basal and upper nodal cells have divided, cf.  $L_1$  of Fig. 4, and therefore like the structure from the upper node of which it has developed. The position this structure occupies suggests that it has developed in place of the second 'leaflet'. From the other node (Fig. 28), in addition to the two 'leaflets', there is a three-celled structure which is exactly like a 'leaf' of the simple type with two cells above the basal nodal cell. The interest of these abnormalities is that they support the view that the 'leaf' of the *N. opaca* type is a form reduced from a type where the upper nodes of the 'leaves' had lateral appendages repeating the structure of the axis bearing them.

In the case of fertile leaves, it is found that usually two of the adaxial cells develop into oogonia, one peripheral cell by divisions forming one oogonium. The number of oogonia present in no way depends on the number of 'leaflets', nor does the opposite hold; the commonest condition is one oogonium and one 'leaflet'.

In no case have all five peripheral cells been found to produce lateral structures and those which do not develop into either 'leaflets' or oogonia divide up into several cells. Although more regular in some than others, on the whole the further divisions are very irregular. The first-formed walls may either cut the cell into two equal or more often unequal portions (cf. Figs. 22, 23, 24), but it appears never to be parallel to the surface wall (Fig. 21,  $p'_3$ ). If it divides the cell into equal halves it is at right angles to

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peripheral cells except one have divided.  $\times 280$ . Figs. 23, 24, 25. Later stages in the segmentation of the peripheral cells. For explanation see text. Figs. 23 and 25.  $\times 160$ . Fig. 24.  $\times 280$ . Figs. 26 and 27. Upper nodes of mature 'leaves' with groups of embryonic cells at the base of (Fig. 26) and between (Fig. 27) the 'leaflets'. The node shown in Fig. 26 is exceptional, for some of the peripheral cells have only divided once.  $\times 160$ . Figs. 28 and 29. Surface view of the upper nodes of two 'leaves' with multicellular 'leaflets'.  $\times 280$ .

$b.n.$  = basal node of 'leaf';  $c.$  = central cell of node of 'leaf';  $c'.$  = daughter cell of central cell;  $i.$  = internodal cell of leaf;  $l.l.$  = 'leaflet';  $p.$  = peripheral cell of node of 'leaf';  $t.s.$  = terminal segment of 'leaf'.

the wall adjoining the central cell and then further walls may be laid down in one or both daughter cells (Figs. 22 and 24). These divisions again may be followed by others, but they are usually confined to the cells with free surface. As before, the new walls may be at right angles to the last (Fig. 23), and then a complex of two inner and four outer cells is built up (Fig. 23). In most cases they are more irregular as the walls are curved and not straight. Even where the first walls are straight the later ones are usually curved. Not only longitudinal but also divisions at right angles to the long axis of the 'leaf' occur, and this is especially the case in the more adaxial peripheral cells, so that the node may be several layers of cells deep at the base of the 'leaflets'. It is also noticeable that the more adaxial peripheral cells divide more than the others. This is shown in Figs. 26 and 27, and it is a marked feature of these cells that they have thinner walls and appear very like embryonic cells.

If the third peripheral cell is formed between the first and second peripheral cells it never seems to develop into a 'leaflet' or oogonium, but usually divides in a rather irregular manner. Like the other more adaxial peripheral cells, the divisions in this cell may be transverse as well as longitudinal, and so at the node of a mature 'leaf' an irregular mass of cells between the leaflets is to be seen (Fig. 27).

It is chiefly the groups of embryonic cells at the base of and between the 'leaflets' which are concerned with the formation of adventitious branches at this node of the 'leaf'. The development of these branches will be described in the next section.

### III. THE OCCURRENCE AND DEVELOPMENT OF ADVENTITIOUS BRANCHES AT THE UPPER NODE OF THE 'LEAF'.

Although adventitious branches are not found so commonly in *Nitella* as in *Chara*, yet they are known to occur at the nodes of the stem (i.e. growing from cells of the basal node of either the 'leaves' or the lateral branches) of *N. gracilis*, *N. syncarpa*, and *N. cernua*.

Richter (11) was unable to obtain pro-embryonic branches from nodes of *Nitella flexilis*, even after four months of isolation, and he therefore concluded they never occur. Adventitious pro-embryonic branches are so far unknown for *N. gracilis* and *N. cernua*, but Giesenhagen (2, 3) records the development of radial branches from cells of the basal node of the lateral branches. In *N. syncarpa*, on the other hand, he found that both radial and pro-embryonic branches may grow from the same node of the stem, although they quite commonly occur separately; this he thinks depends on whether the plant is young or older and more strongly developed. He found that nodes of plants grown indoors throughout the winter all formed rhizoids.

and adventitious shoot apices, but these apices seldom occurred in the axils of the youngest 'leaves'.

These records deal with the development of adventitious branches from the basal nodes of 'leaves', and the only case known of such branches developing from any other node, such as one of the upper nodes of a 'leaf', is a figure of Giesenhagen's (Flora, lxxxiii, 1897, Taf. v, Figs. 5, 6, 7) of a 'leaf' of *Nitella* (sp. not given), showing a radial branch growing from such a node. There is no mention of it in the text, but Goebel (4) in describing it adds that after the shoot had been cut off the young rhizoid rudiments at its base developed into pro-embryonic branches.

This author holds the view that the original shoot which was cut off had replaced an oogonium. This is certainly not the explanation of the appearance of the adventitious branches which are to be described growing from the upper nodes of 'leaves' of *N. opaca*, for they may be stimulated to develop from nodes, where previously there was no sign of either an oogonium or apex. They also are formed in winter, when the plants are sterile, as well as in summer, from 'leaves' also bearing oogonia.

*Nitella opaca* has been used for all the experiments to be described. Although it is especially the development of adventitious branches from the upper node of the 'leaf' with which this paper deals, yet it should be stated that, as in other Characeae, adventitious branches also develop readily from the basal nodes of the 'leaves' (Fig. 30). There appear to be no accessory branches in *N. opaca*.

Mere isolation of a node by cutting through the neighbouring inter-nodal cells is occasionally sufficient to stimulate the development of adventitious branches from the basal nodes of the 'leaves', but they develop with greater certainty if the lateral branches are cut off as well. The adventitious branches are both radial and pro-embryonic, and develop quite soon after the isolation of the node, for they are often visible to the naked eye within a fortnight. Both types of branch may grow from the same node (Fig. 30, *pr.* and *r.br.*), yet some nodes may produce one type only, and, unlike *Chara vulgaris*, the adventitious branches are not limited to any one part of the node, but are found in the axils of the youngest as well as the oldest 'leaves'. Sometimes these branches are found in quite large numbers; twenty-four were counted at one node. Although no distinction can be drawn between 'nacktfüssige' and normal radial branches in the case of *Nitella*, yet the adventitious branches often show a simpler construction than the lateral branches. The 'leaves' of the older nodes are not only fewer, but they are either noded without 'leaflets' or nodeless. In extreme cases the 'leaves' remain rudimentary, appearing as globular cells at the node. This is a simplification parallel to the 'nacktfüssige' branches of the corticated Characeae.

Adventitious branches not only grow from the basal nodes of 'leaves'

of *N. opaca* under experimental conditions, but quite often old nodes of plants growing in their natural habitat are found with several adventitious

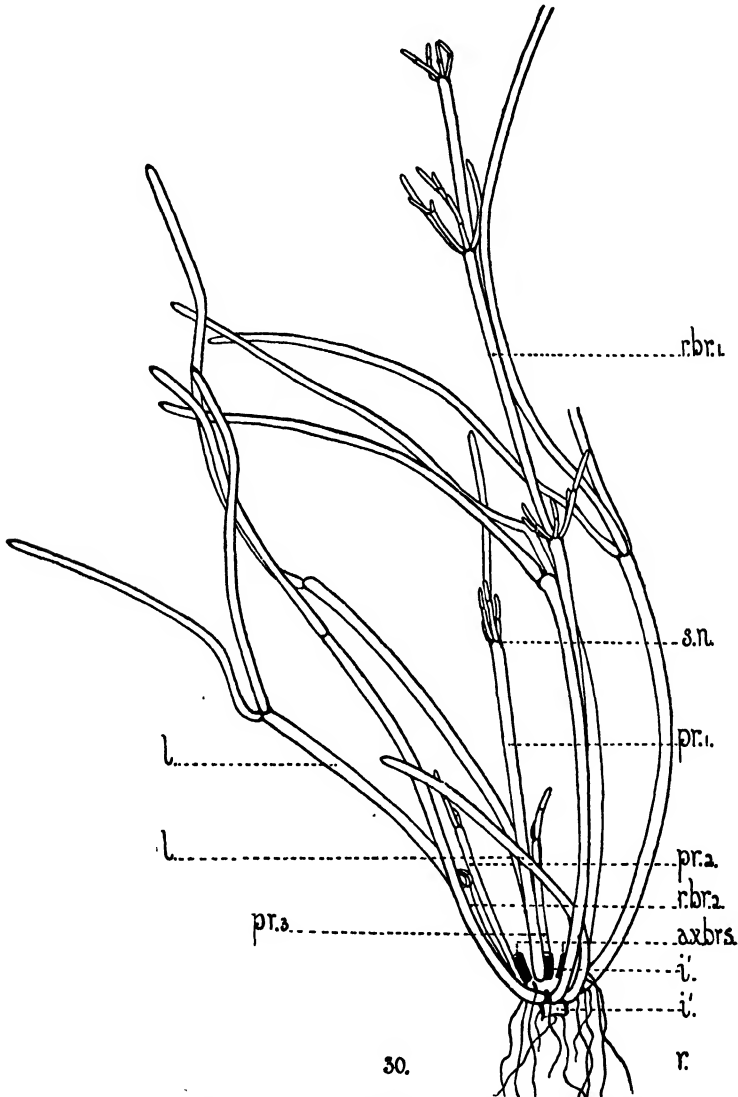


FIG. 30. Adventitious radial (*r.br.*) and pro-embryonic (*pr.*) branches growing from an isolated node of *N. opaca*. The axillary branches had been cut off (*ax.brs.*).  $\times 4$ .

*ax.brs.* = axillary branches; *i.* = internodal cell; *l.* = 'leaf'; *pr.* = pro-embryonic adventitious branch; *r.* = rhizoid; *r.br.* = radial adventitious branch; *s.n.* = stem-node of pro-embryonic branch.

branches, both radial and pro-embryonic. It is surprising that branches do not appear at the upper node of the 'leaf' in nature also, since it is so easy to stimulate their development under experiment. The difficulty

of finding examples in nature may account for the absence of such records.

Development of adventitious branches from the upper node of the 'leaf' has been stimulated by subjecting isolated whorls, or in other cases isolated 'leaves', to various experimental conditions. These experiments, which have been carried out at all times of the year, can be grouped thus:

- A. Isolation of nodes of the shoots of unlimited growth, i.e. the main axis or the lateral branches.
- B. Isolation of nodes of the shoots and injury to the 'leaves'.
- C. Separation of the 'leaves' with and without the basal node.

In all cases, after isolation, each node of the shoot or each 'leaf' was grown separately on a layer of sand in a dish or test-tube of tap-water.

*A. Isolation of Nodes of the Shoots of Unlimited Growth.*

By cutting through the internodal cells, shoots of *N. opaca* were divided into pieces, each consisting of one node. In some cases the lateral branches were left uninjured, but either both or one of the branches was cut off just above the basal node, from other whorls.

If both of the lateral branches are cut off, in addition to the adventitious branches which develop from the basal nodes, some also grow from the upper node of some of the 'leaves'. If only one branch is removed there is a decrease in the number of 'leaves' from which adventitious branches develop, and adventitious branches seldom develop from the upper node of the 'leaf'. If neither of the lateral branches are cut off, then adventitious branches seldom appear, even from the basal nodes of the 'leaves', the existing branches taking the place of the main apex. Although the 'leaves' were not injured at the beginning of the experiment, a certain number were either quite dead or the internodal cell or the 'leaflets' were dead at the end. In many cases, however, adventitious branches had grown from the upper node of these 'leaves'.

*B. Effect of Injury to the 'Leaves'.*

Since adventitious branches were often found growing from partially or in rare cases completely dead 'leaves', some 'leaves' were injured in order to see what effect it would have on the formation of these branches. Nodes of a shoot were isolated, as already described, and the internodal cell between the upper and basal nodes of each 'leaf' of the whorl pricked with a dissecting needle.

These experiments have given the best results, for adventitious branches develop from some 'leaves' of almost every isolated whorl. In some cases

adventitious branches grew from the upper node of every 'leaf' of the whorl. The internodal cell ultimately dies, as a result of the injury, and the 'leaf' then falls to the sand and, by a development of rhizoids from the upper node, fixes itself to the sand very firmly. From such nodes there is a prolific development of adventitious branches, which may be radial (i. e. like the main axis) or pro-embryonic.

Instead of pricking the internodal cell of 'leaves' of isolated whorls, the basal nodes were pricked with a dissecting-needle. The cells of these nodes then die, and so the 'leaves', which are otherwise still healthy, are frequently found isolated at the end of the experiment.

This is a fairly certain method of stimulating branch development from the upper node of the 'leaf', for many 'leaves' react when treated in this way, but not such a high percentage as when the internodal cell is injured.

The following table gives the results obtained from a series of twelve nodes, as alike as possible, the internodal cell of 'leaves' of six nodes being injured (I) and the basal nodes of the 'leaves' of the other six (II):

I				II			
No.	No. of 'leaves' with nodes.	No. of 'leaves' with adv. brs.	%	No.	No. of 'leaves' with nodes.	No. of 'leaves' with adv. brs.	%
1.	6	4	66.6	1.	8	3	37.5
2.	8	5	62.5	2.	7	6	75.8
3.	8	7	87.5	3.	7	2	28.6
4.	7	7	100	4.	8	1	12.5
5.	6	3	50	5.	8	4	50.0
6.	6	1	16.6	6.	7	4	57.2

If the 'leaf' is injured more severely by cutting off the 'leaflets' and terminal segment of each 'leaf' of the isolated whorls, adventitious branches do not develop from the upper node of many of these 'leaves'.

### C. Separation of the 'Leaves' from the Nodes of the Branches.

If 'leaves' are separated from the main axis by cutting through the internodal cell between the basal and upper nodes, the 'leaf' soon dies, and only exceptionally do adventitious branches develop. This is a striking contrast to the results obtained by isolating 'leaves', each with its basal node, for then adventitious branches develop from the upper node of many of the 'leaves'. After isolation of the 'leaf' the cells of the basal node usually die and decay, but the rest of the 'leaf' remains alive.

In one experiment ten pairs of 'leaves' were isolated, the 'leaves' of each pair being as alike as possible and separated from the same node. The basal node of one 'leaf' of each pair was removed with the 'leaf', whereas the other 'leaf' was separated from the rest of the whorl by cutting through the internodal cell. Most of the 'leaves' separated by cutting the internodal cell died very quickly, and only three showed any

branch development, but only two 'leaves' of the other series died, and of the remaining eight, seven showed abundant branch development within three weeks.

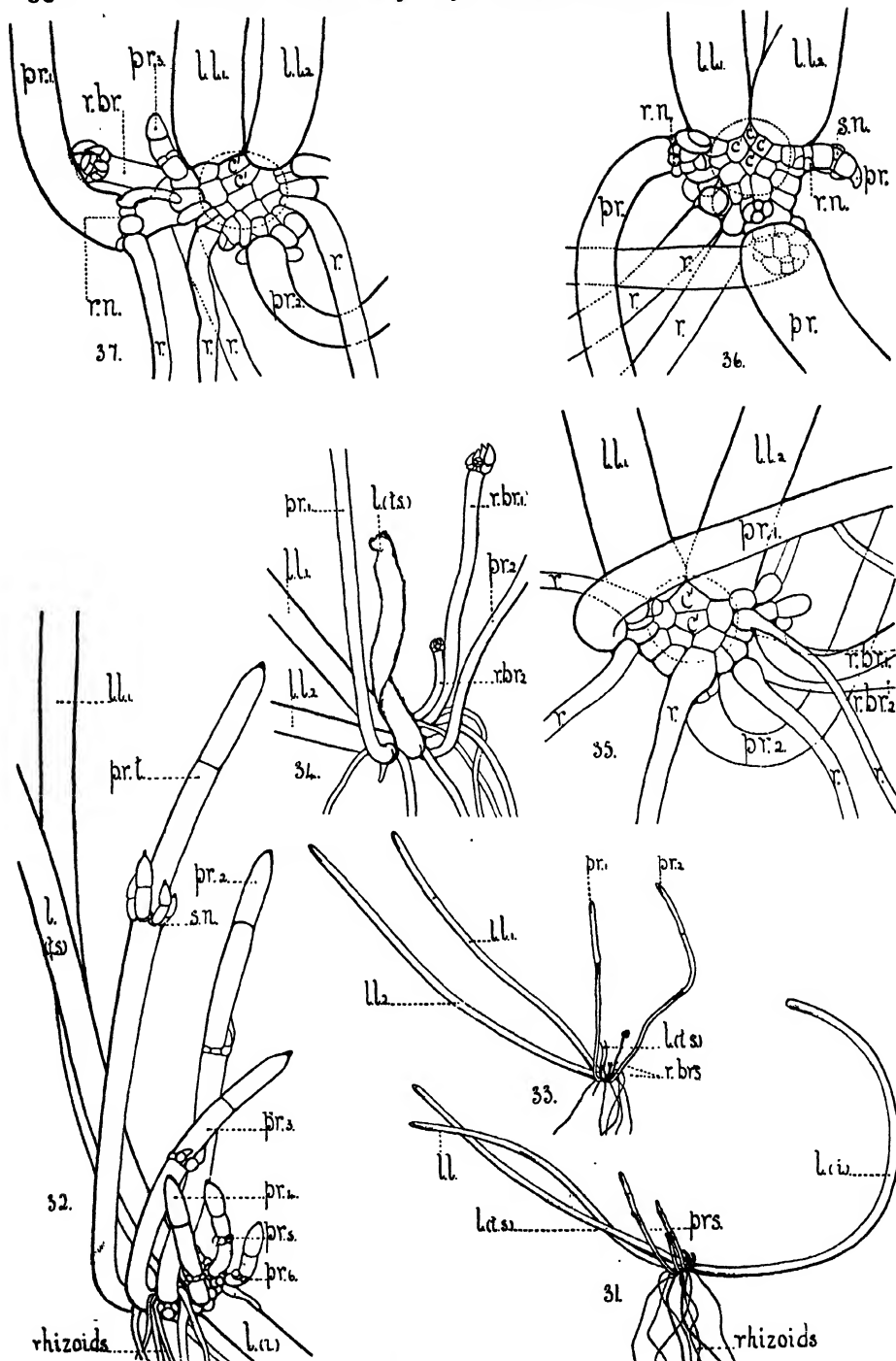
The results of these experiments have shown quite clearly that adventitious branch development is much more abundant under some conditions than under others, although branches have developed at some time or other from 'leaves' in all the experiments described.

Throughout the experiments it has become obvious that the first and often the most prolific development of adventitious branches from the upper node of the 'leaf' is near the base of the 'leaflets'—that is, on the adaxial side of the node. This is evidently correlated with and probably the result of the more active cell division which is found at the base of the 'leaflets' (cf. Figs. 26, 27) and the presence of embryonic cells at that part of the node. If the first branch is formed at the base of one of the 'leaflets' others may follow at its base, as in Figs. 31 and 32—a 'leaf' which had been separated with its basal node from the node of the stem. On the other hand, the next branches may be formed at quite a different part of the node, e.g. Figs. 34, 35. In this case the basal node of the 'leaf' was injured at the beginning of the experiment, and during the experiment the internode and terminal segment of the 'leaf' died also. If isolated 'leaves' are laid on sand, the branches develop chiefly from the side away from the light.

Nodes from which adventitious branches have developed are seen in surface-view in Figs. 35, 36, 37, and 41. By treatment with Eau de Javelle, the node with the branches can be separated from the rest of the 'leaf'. The basal node of the 'leaf' shown in Fig. 35 had been injured, but during the experiment the internode and terminal segment died also. The part of the 'leaf' which remained with the branches is seen in Figs. 33 and 34. Two radial branches (*r.br.*<sub>1</sub> and <sub>2</sub>) grew from the daughter cells of one of the peripheral cells and a pro-embryonic branch (*pr.*<sub>1</sub> and <sub>2</sub>) from cells derived from each of the other two peripheral cells.

Fig. 36 is of the upper node of a 'leaf' the internode of which had been injured. One pro-embryonic branch (*pr.*) grew from each group of cells, derived from the three peripheral cells, which did not develop into 'leaflets'. One of these branches is very much younger than the other two.

Radial and pro-embryonic branches not only develop from cells of the node of the 'leaf' direct, but also quite commonly from the basal nodes of other radial and pro-embryonic branches. In Fig. 37, which is the upper node of a 'leaf', the internode of which had been injured, a radial (*r.br.*) as well as a pro-embryonic branch (*pr.*<sub>3</sub>) are seen to have developed from the basal node of an older pro-embryonic branch (*pr.*<sub>1</sub>). In addition, another pro-embryonic branch (*pr.*<sub>2</sub>) has grown from a more abaxial cell.



FIGS. 31-7. Adventitious branch development from the upper node of the 'leaf' of *Nitella opaca*.



Adventitious branches may develop between the 'leaflets' (Fig. 41) in the case of nodes, where the third peripheral cell developed in that position as described.

Branches not only grow from nodes bearing oogonia (Fig. 49), but even between an oogonium and 'leaflet' or two oogonia.

Experiments have been carried out at practically all times of the year, and although there is no marked difference in the number of branches formed in the winter and summer, yet there is a striking difference in the type of branch formed at the upper node of the 'leaf'. The adventitious branches may, like those which are known to develop from the basal node of the 'leaf', be either of the radial type (Fig. 48)—that is, like the shoots of the mature plant—or they may be pro-embryonic (Figs. 31, 32). Although both types of branch may develop from the same node throughout the year (Figs. 33, 34, 35), during the winter months practically all the adventitious branches are pro-embryonic, whereas in summer the majority are radial:

<i>Dates of Expts.</i>	<i>No. of 'Leaves' with Branches.</i>	<i>Ratio of Radial to Pro-embryonic Branches.</i>	<i>%</i>
1. Jan., Mar., April, . Oct., Nov., Dec.	86	37:175	17.5:82.5
2. May, June, July	20	31:6	84:16

This table includes far more experiments in the winter than the summer months, but the same results are obtained if more equal periods are taken.

The difference in the type of branch which predominates in winter and summer is no doubt influenced by external conditions, which are more favourable in summer and less favourable for growth in winter.

So few of the pro-embryonic branches have a rhizoid-node (cf. Fig. 32) that it can hardly be treated as an abnormality, rather as a point of difference between the pro-embryo of the spore and the adventitious pro-embryonic branch. Otherwise the two structures are alike. The 'leaves' of the stem-node are often of a very simple type, consisting of two cells only above the basal node, and only those on the side of the node where

Fig. 31. Pro-embryonic branches (*pr.*) growing from the upper node of an isolated 'leaf'.  $\times 3$ . Fig. 32. Node of 'leaf' shown in Fig. 31.  $\times 17$ . Fig. 33. Radial (*r.br.*) and pro-embryonic (*pr.*) branches growing from the upper node of an injured 'leaf'. The internodal cell has decayed and only a fragment of the terminal segment (*t.s.*) remains.  $\times 3$ . Fig. 34. Node of the same 'leaf'.  $\times 7$ . Fig. 35. Node of the same 'leaf' in surface view, showing the place of origin of the branches.  $\times 50$ . Fig. 36. Surface view of the upper node of a 'leaf', the internode of which had been injured, showing the place of origin of adventitious pro-embryonic branches.  $\times 50$ . Fig. 37. Upper node of another injured 'leaf', showing adventitious branches developing from the basal node of an older pro-embryonic branch.  $\times 50$ .

*c.* = daughter cell of central cell of the upper node of the 'leaf'; *l.(i.)* = internodal cell of the 'leaf'; *l.(t.s.)* = terminal segment of the 'leaf'; *l.l.* = 'leaflet'; *pr.* = pro-embryonic branch; *pr.t.* = pro-embryo-tip; *r.* = rhizoid; *r.br.* = radial branch; *r.n.* = rhizoid-node of pro-embryonic branch; *s.n.* = stem-node of pro-embryonic branch.

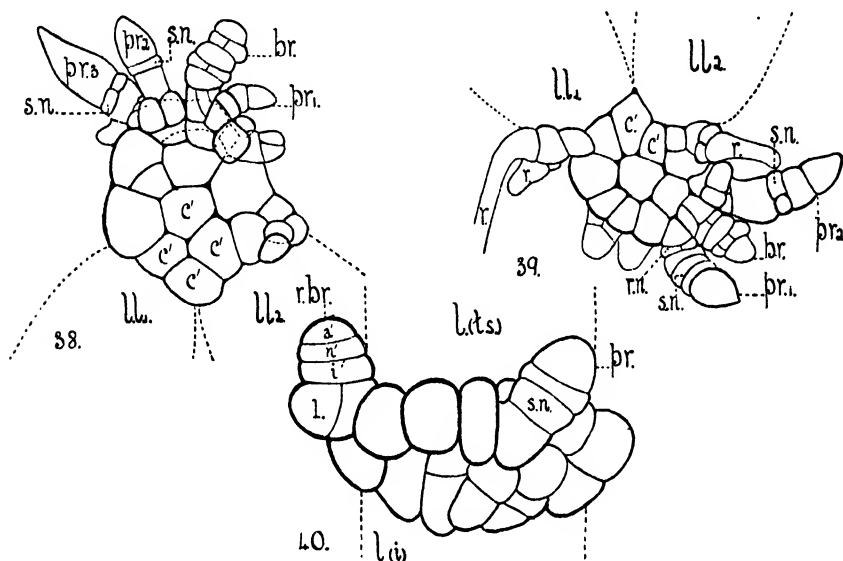
the branch apex is formed reach any size (Fig. 32, *s.n.*). The peripheral cells on the opposite side may never divide.

Young stages in the development of adventitious branches are represented in Figs. 38, 39, 40, which also show quite clearly that one single cell develops into a branch. Fig. 38 is the node of an uninjured 'leaf' of an isolated node of the main axis, and Figs. 39 and 40 of 'leaves' the internodal cell of which had been injured.

The pro-embryonic branch, marked *pr.*<sub>1</sub> in Fig. 38, consists of a row of four cells, the result of divisions in one of the outermost cells of the 'leaf'. The innermost of these cells will form the basal node of the branch, and the next is the segment-cell from which the stem-node and the internode immediately below (and possibly the rhizoid-node) will develop. The two outermost cells will become the pro-embryo-tip. Slightly older stages of development are shown in this same figure, but both branches are abnormal, as each has only one cell in the pro-embryo-tip. In the one (*pr.*<sub>2</sub>) the stem-node has not divided, and it has in the other (*pr.*<sub>3</sub>). The change in shape of the apical cell is also clearly brought out. The apical cell of the branch *pr.*<sub>3</sub> is much more mucronate and elongated than that of the branch marked *pr.*<sub>1</sub>. Similar stages are seen in Fig. 39, but here the pro-embryonic branches are quite normal, with two cells in the pro-embryo-tip, and also they each have a root node (*r.n.*). The stem-node (*s.n.*) of one branch (*pr.*<sub>1</sub>) has not divided, and one division has taken place in the stem-node of the other (*pr.*<sub>2</sub>). Growing from both nodes figured are branches which show how alike radial and pro-embryonic branches can be in very young stages. It is impossible to tell into which type of branch those marked *br.* will develop.

Earlier stages than these are uncertain, as small two-celled structures of uncertain nature are quite common amongst the branches, especially in summer, and there seems no way of distinguishing them from young pro-embryonic branches. In Fig. 40, however, the nature of the embryonic branches can be determined with safety. The upper node of the 'leaf' is viewed from the side and two embryonic branches are seen. One is pro-embryonic (*pr.*) and consists of four cells, the two cells of the pro-embryo-tip, the stem-node (*s.n.*), and a lower cell. The other is a young radial branch, and the apex (*a*) has cut off one segment cell which has divided. The basal node has also divided, and is of special interest, for the peripheral cell (*l.*), by its position and shape, is very like the 'leaf' initial which occurs at the base of the lateral branches of the main axis. The size and complexity of the basal node of the adventitious pro-embryonic branches varies very much, probably depending to some extent on the size of the cell from which the branch develops. Two very large pro-embryonic branches grew from the node shown in Figs. 42 and 43. Both were abnormal (see Figs. 44 and 45), one (the smaller) in that the pro-embryo-tip was unicellular and the

other as it had formed two stem-nodes ( $s.n._1$  and  $s.n._2$ ). The terminal cell of the 'leaf' and the 'leaflets' had decayed away before the end of the experiment; and it was not surprising, therefore, to find the node itself incomplete. On the one side of the node (cf. Fig. 42) the basal nodes of the two branches ( $pr._1$  and  $pr._2$ ) are conspicuous, and it can be seen that each consists of a central cell ( $c.c.$ ) around which there are numerous cells,



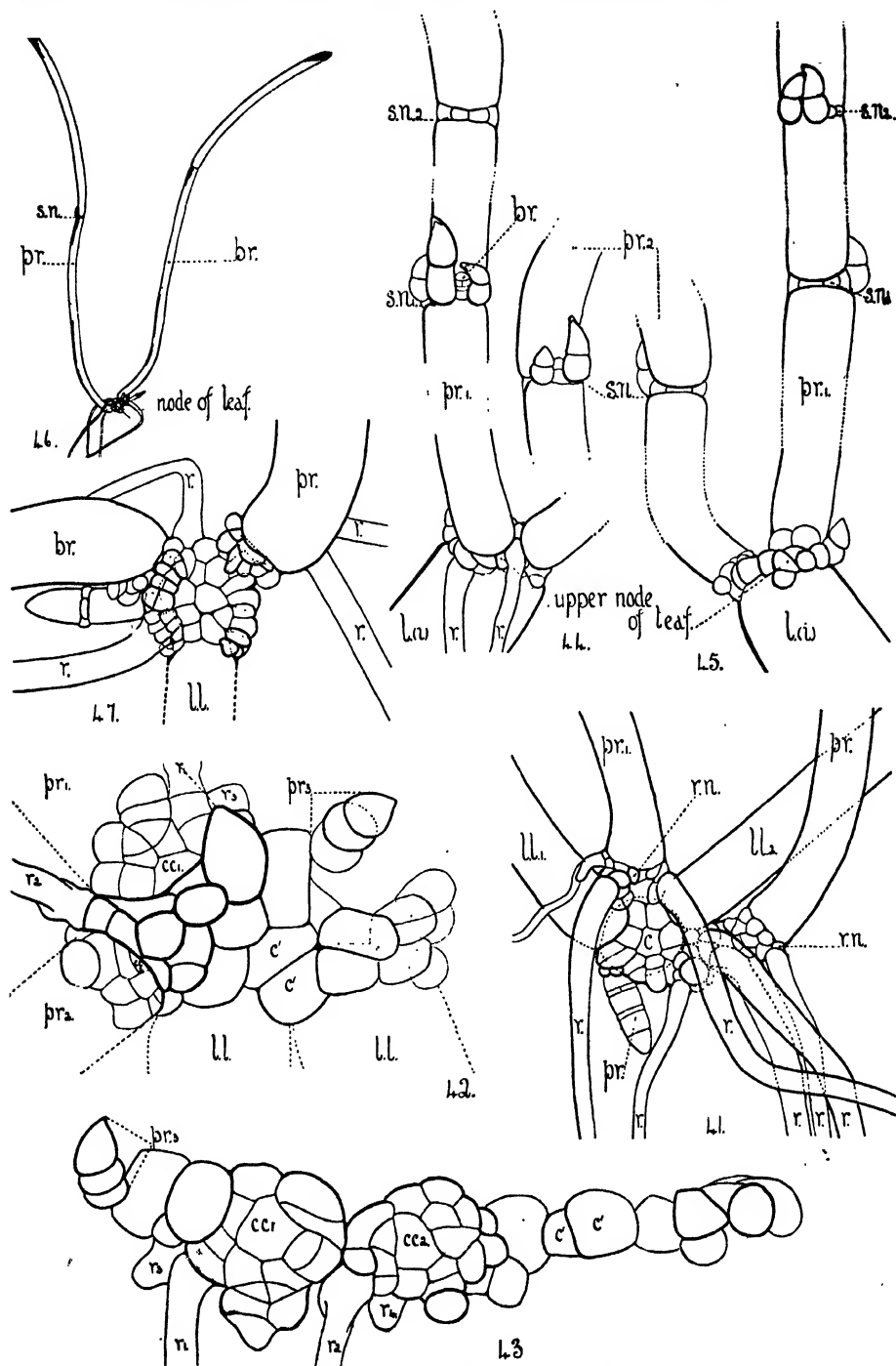
FIGS. 38-40. Young stages in the development of adventitious branches. Fig. 38. Upper node of 'leaf' in surface view, showing stages in the development of pro-embryonic branches ( $pr._1$ ,  $pr._2$ ,  $pr._3$ );  $pr._2$  and  $pr._3$  are abnormal, as the pro-embryo-tip is unicellular. Fig. 39. Also a surface view of a similar node with young pro-embryonic branches, showing the stem-node ( $s.n.$ ) and rhizoid-node ( $r.n.$ ).  $\times 75$ . Fig. 40. Young radial ( $r.br.$ ) and pro-embryonic ( $pr.$ ) branches growing from cells of the upper node of a 'leaf', seen from the side.  $\times 16c$ .

$a'$  = apical cell of radial branch;  $br.$  = branch;  $c'$  = daughter cell of central cell;  $L(i)$  = internodal cell of 'leaf';  $L(t.s.)$  = terminal segment of 'leaf';  $L.l.$  = 'leaflet';  $i'$  = internodal cell of radial branch;  $n'$  = nodal cell of radial branch;  $pr.$  = pro-embryonic branch;  $r.$  = rhizoid;  $r.n.$  = rhizoid-node of pro-embryonic branch;  $s.n.$  = stem-node of pro-embryonic branch.

the result of divisions in the peripheral cells. This is more clearly seen in Fig. 43, which shows the node from the side, if split by the small two-celled structure. It is not surprising that the peripheral cells of basal nodes such as these give rise to further adventitious branches, as indeed frequently happens.

In addition to adventitious branches without a root-node and with only one cell instead of two, in the tip of the branch—irregularities of such frequent occurrence that they can hardly be called abnormal—other abnormalities are found.

In some cases the pro-embryonic branch has two nodes, each bearing 'leafy' structures and representing a stem-node (Figs. 44 and 45,  $s.n._1$  and  $s.n._2$ ). There are two possible explanations: either the rhizoid-node has



FIGS. 41-7. Fig. 41. Upper node of a 'leaf' in surface view, showing a pro-embryonic branch (*pr.*) developing from cells between the 'leaflets' (*ll.* and *ll.*) as well as from cells on the abaxial side

become a stem-node, or else one of the segment cells forming the pro-embryo-tip has divided into a node and internode.

The latter is more probable, since these branches seldom have a rhizoid-node, and also whenever there are two such nodes there is only one cell above the upper node, suggesting, therefore, that the other has divided.

Among the adventitious branches, during the summer especially, there are often outgrowths, consisting of two cells, placed end to end, with or without a basal node, and of very varying size. By their position in relation to a radial branch some of these must be considered as 'leaves' equivalent to the secondary 'leaves', which develop from the basal node of the lateral branches of the main axis (Figs. 40 and 49). Many, however, do not develop in relation to a branch (Fig. 54), and it is then difficult to determine whether the structure is a modified 'leaf' or an arrested pro-embryonic branch. Fig. 46 illustrates the resemblance between such a structure (*br.*) and a pro-embryonic branch (*pr.*). The upper node of the 'leaf' from which these two outgrowths had developed is shown in surface view in Fig. 47. These two branches had developed from daughter cells of two of the abaxial peripheral cells, and one consists of two elongated cells above its basal node, whereas a node separates the two corresponding cells of the other branch (Fig. 46). There are a few embryonic 'leaves' at this node (*s.n.*). The first of these structures (*br.*) is exactly like a simple 'leaf' and the second a pro-embryonic branch without a rhizoid-node and only one instead of two cells above the tip. These two branches have developed in exactly the same way, except that the segment cell of one has divided into node and internode, and in the other it has not. This division has made one a pro-embryonic branch and left the other a simple structure which may be considered as either a simple 'leaf' or an arrested pro-embryonic branch.

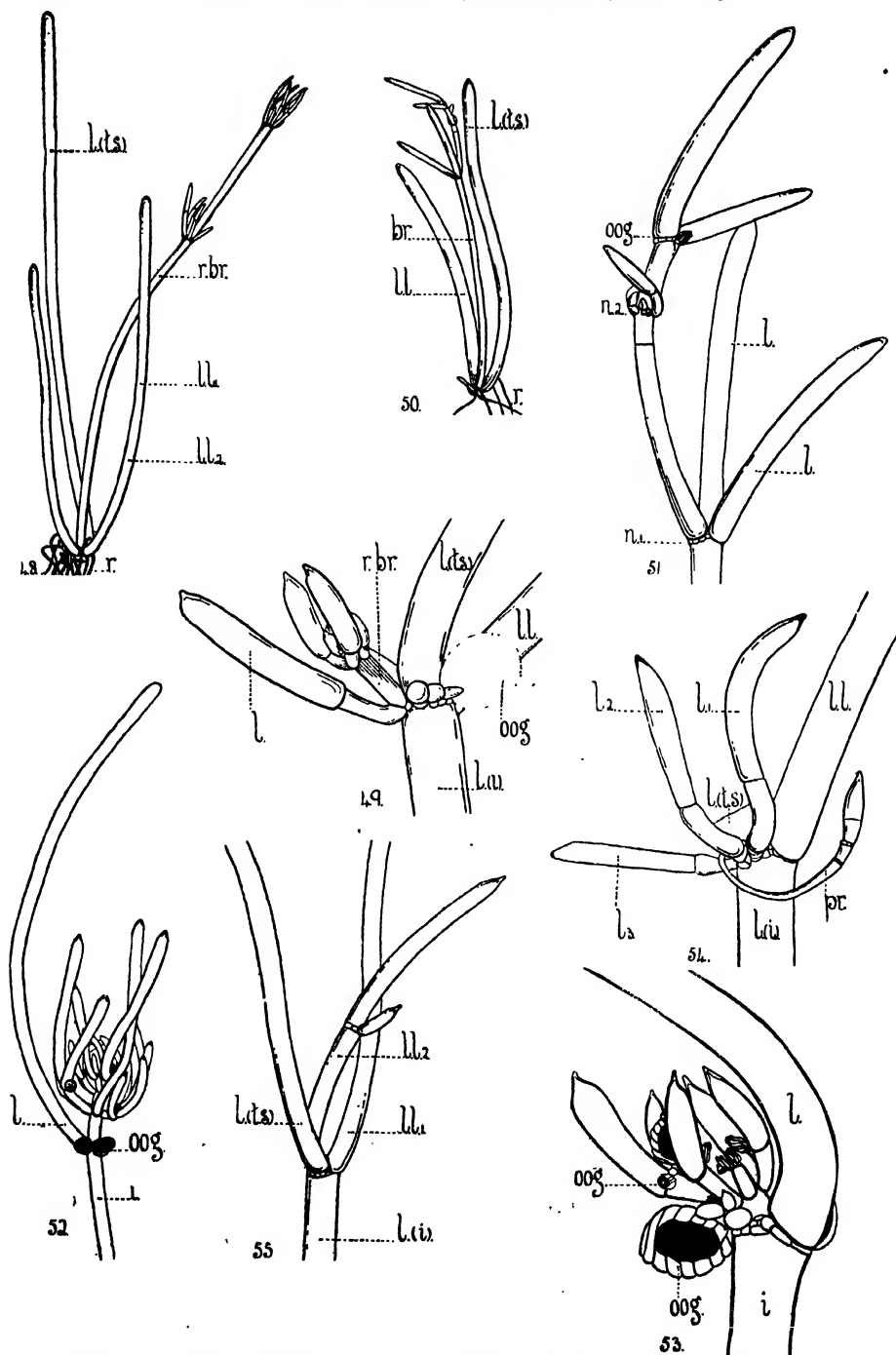
Before the lateral branch of pro-embryonic branches, such as this one (Figs. 46 and 47, *pr.*) appears, they are very leaf-like in appearance, the only difference being in the complexity of the lateral appendages at the node. This suggests that the pro-embryo has arisen by a simplification somewhat in the same way as a 'leaf'.

One of the very first stages in the development of a radial adventitious

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of the 'leaf'.  $\times 50$ . Fig. 42. Upper node of a 'leaf' (incomplete) showing the basal nodes of two pro-embryonic branches (*pr.*<sub>1</sub> and *pr.*<sub>2</sub>).  $\times 107$ . Fig. 43. Same node from the side. For explanation see text.  $\times 107$ . Figs. 44, 45. Upper node of same 'leaf' showing the two pro-embryonic branches, which are both abnormal—*pr.*<sub>1</sub> has two stem-nodes (*s.n.*<sub>1</sub> and *s.n.*<sub>2</sub>) and *pr.*<sub>2</sub> has only one cell above the stem-node.  $\times 50$ . Fig. 46. Two abnormal branches growing from the upper node of an injured 'leaf'.  $\times 5$ . Fig. 47. Same node enlarged to show the place of origin of the branches.  $\times 50$ .

*br.* = branch; *c.* = central cell of upper node of the 'leaf'; *c'.* = daughter cell of central cell of upper node of the 'leaf'; *c.c.* = central cell of basal node of adventitious branch; *l.(i.)* = internodal cell of the 'leaf'; *l.l.* = leaflet; *pr.* = pro-embryonic branch; *r.n.* = rhizoid-node of pro-embryonic branch; *r.* = rhizoid; *s.n.* = stem-node of pro-embryonic branch.



FIGS. 48-55. Fig. 48. Radial branch (*r.br.*) growing from the upper node of a 'leaf'. The internode below this node has decayed.  $\times 3$ . Fig. 49. Young radial branch (*r.br.*) growing from the

branch has already been figured and described (Fig. 40, *r.br.*). The basal node of that branch has divided into two, one of the daughter cells protruding (*l.*). By comparison with young stages in the development of the lateral branches of the main shoot it is concluded that this cell will develop into a 'leaf'. An older branch (*r.br.*) subtended by a 'leaf' is shown in Fig. 49. The 'leaf' has no node above the basal node, and in this respect is like many of the secondary 'leaves' of the nodes of the main shoot. On account of their position there is no doubt that such two-celled outgrowths are 'leaves', and must be considered as equivalent to secondary 'leaves', although in other cases, as it has been pointed out, they may be arrested pro-embryonic branches.

Many of the first 'leaves' of the adventitious radial branches are quite simple, i.e. they have no node above the basal node. None of the 'leaves' of the first and second nodes of an older branch (*r.br.*) shown in Fig. 48 have 'leaflets'. Sometimes some of the 'leaves' of the older nodes do not develop, although the peripheral cell may enlarge slightly. These simplifications may be treated as parallel to those described for the corticated Characeae resulting in 'nacktfüssige' branches.

Occasionally, abnormal branches develop from the upper node of the 'leaf'. One of the most interesting is represented in Figs. 50 and 51. In general appearance it is like a 'leaf' the terminal segment of which has been replaced by a branch apex. The oldest node is just like the upper node of a 'leaf', for two unicellular outgrowths (*l.*) resembling 'leaflets' are the only structures growing from it. There are no lateral branches, and the other peripheral cells have divided like those of the corresponding node of a 'leaf'. The internode which separates this node and the next is divided into an upper smaller and lower larger cell. The upper node is still very young, and has only six 'leaves' which are of very varying size. Variation in the size of the 'leaves' of the first nodes, even when mature, of adventitious radial branches is not at all unusual.

Similar structures, having characters of both a 'leaf' and a branch, have been found on material collected from two widely separated localities. Two are shown in Figs. 52 and 53. Excepting the first node, the branch in Fig. 52 is quite normal. There is only one 'leaf' (*l.*), however, at the

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upper node of a 'leaf' which was bearing oogonia (*oog.*). The branch is subtended by a 'leaf' (*l.*) × 7. Fig. 50. Abnormal branch (*br.*) developing from the upper node of a 'leaf'. The internodal cell of the 'leaf' has decayed. × 3. Fig. 51. Nodes of the abnormal branch enlarged. × 17. Fig. 52. Abnormal 'leaf'. For explanation see text. × 4. Fig. 53. Abnormal 'leaf'. For explanation see text. × 27. Fig. 54. Adventitious 'leaf-like' outgrowths (*l.*) from the upper node of a 'leaf'. A pro-embryonic branch has also grown from this node. The terminal segment of the 'leaf' has decayed. × 27. Fig. 55. Node of a 'leaf' with a branched 'leaf'-like 'leaflet' (*l.l.*) × 17.

*br.* = adventitious branch; *i.* = internodal cell; *l.* = 'leaf'; *l.(i.)* = internodal cell of 'leaf'; *l.l.* = leaflet; *l.(l.s.)* = terminal segment of 'leaf'; *n.* = node; *oog.* = oogonium; *pr.* = pro-embryonic branch; *r.* = rhizoid; *r.br.* = radial branch.

first node, and that is unicellular and therefore like a 'leaflet'. In addition, there are three oogonia (oog.). Judged by the nature of the structures growing from it, the oldest node is like the node of a 'leaf' and not a branch. The structure represented in Fig. 53 replaced a 'leaf' at the node of the main shoot. Unlike a 'leaf', it is terminated by an actively dividing dome-shaped apical cell. At the stage figured there were four nodes, two hidden inside the 'leaves' of the third, which with the oldest is shown in the figure. The third node is like that of any lateral branch, excepting that there are only six 'leaves', and no branch apices can be distinguished. The oldest node is very like the oldest node of the branch in Fig. 52, for two oogonia (oog.) and a unicellular 'leaf' or 'leaflet' are the only structures growing from it. In this case the 'leaflet' has a basal node. Between the 'leaflet' and one oogonium are two groups of cells and another group between the two oogonia, evidently formed, by division, from the peripheral cells. This node, like the oldest node of the other branch, is much more like the upper node of a 'leaf' than a node of a branch. Both of these branches, therefore, are like a 'leaf' in the structure of the oldest node, but unlike a 'leaf' by the continued growth and division of the apical cell.

Without following the development of structures such as these it is impossible to decide whether they are radial branches, the oldest node of which is modified, or 'leaves', the apical cell of which has remained active. But the interest lies rather in the fact that they are intermediate in structure, between a branch and a 'leaf', showing that the 'leaf' of the Characeae may have been derived by simplification from a structure like the lateral branch.

#### IV. SUMMARY AND CONCLUSION.

On the whole, the Characeae is a remarkably uniform group of plants, the chief variation being in the construction of the 'leaves'. The small group of species to which *Nitella opaca* belongs is characterized by a very simple type of 'leaf' and also by the development of at least two lateral branches at each node of the axis, there only being one in the other Characeae.

Each branch is lateral to, and not in the axil of, the 'leaf' from the basal node of which it develops, and from the basal node of each branch a secondary 'leaf' is formed. Since the secondary 'leaves' occupy a position between the other 'leaves' of the whorl, they look like the primary 'leaves'. This arrangement of the 'leaves' and branches at the node agrees with Giesenhagen's (2) description for *Nitella syncarpa*.

The 'leaf' of *N. opaca* is extremely simple and of interest when compared with other types found in the family. The apical cell cuts off two segment cells (Fig. 4,  $l_2$ ) only, so that, in addition to the basal node, the



mature 'leaf' has only one node, above which is a long green segment, the modified apical cell of the 'leaf' (Fig. 4,  $L_1$ , *t.s.*).

Under normal conditions no structures develop from the basal node, but two or three unicellular 'leaflets', which closely resemble the modified apical cell, develop from the adaxial side of the upper node. Both nodal cells of the 'leaf' are essentially alike in their development. In each, peripheral cells are cut off from a central cell without a previous division of the node into two halves, and therefore differing from the nodal cell of the main axis. The ring of peripheral cells may never be completed in the basal node as it is in the upper, and the two nodes of the 'leaf' also differ, in that 'leaflets' develop from the cells of the upper node, but never from those of the basal node. These differences between the two nodes are possibly due to the position of the basal node between the two neighbouring internodal cells of the main axis, and there is no reason for regarding it as anything but a modified node of the 'leaf'. In some species, as Ernst (1) has shown in *N. hyalina*, stipular 'leaves' (Stipularblätter) develop from the basal node and resemble, exactly, the 'leaflets' of the next node of the 'leaf'. On those grounds that author claims that the basal node is only a modified node of the 'leaf'. At the same time he asserts that all stipules in the Characeae are to be considered as specialized 'leaflets'.

There is considerable range in structure of the 'leaf' in the Characeae, and a comparative study suggests how the more extreme types such as *N. opaca* have been derived, and also that the 'leaf' is a modified branch of limited growth.

In the majority of the species of *Nitella* and in other genera the apical cell of the 'leaf' cuts off more segment cells than in *N. opaca*. In *N. hyalina*, for example, each of these may divide to form a node and internode, but in most species of *Chara* only the basal ones do so. The 'leaflets' formed at the node may be unicellular, as in *N. opaca*, or multicellular, but without nodes, e.g. *Tolypella*. In *Chara* and *Nitellopsis* the 'leaflets' have basal nodes, and in many species of *Nitella* they not only have basal nodes, but, in addition, other nodes from which further 'leaflets' originate. This type of 'leaf' is very branch-like, the only essential difference between it and a branch being in the limited number of divisions of the apical cell, which ultimately elongates and becomes the terminal segment of the 'leaf'. One feature of this type of 'leaf' is that the number of the 'leaflets' decreases from the older to the younger nodes and that the 'leaflets' at these nodes are progressively simpler. Although the *N. opaca* 'leaf' appears simpler than the others, it seems probable, therefore, that it is a form derived from a more complex type, which was more branch-like. Sometimes 'leaflets' with nodes develop on 'leaves' of *N. opaca*, and this also supports this view.

The 'leaf' of the Characeae seems to have been developed by

simplification of a structure resembling the main axis, firstly by the limited growth of the apical cell and then by further specializations characteristic of the different genera.

It has been known for some time that the cells of the basal nodes of the 'leaves' of many of the Characeae, which do not develop into the so-called stipules or cortical filaments, divide up, somewhat irregularly, and that some of these cells may develop into adventitious branches, and in some cases 'leaves'. This occurs not uncommonly in nature, and can be brought about quite easily by experiment.

It has been found that, unlike the forms described by Pringsheim (9, 10), Giesenhagen (2, 3), and others (6, 11), under certain conditions adventitious branches may be stimulated to develop just as readily from the upper as from the basal nodes of the 'leaves' of *N. opaca*.<sup>1</sup>

As in all questions of regeneration, the exact causal factors are difficult to determine. The problem is to some extent simpler than it would be in a more highly differentiated plant. Besides, *N. opaca* being a comparatively simply constructed organism, most cell divisions are regular and limited. In addition, at every node there already exist groups of embryonic cells.

The conditions under which these cells can be made to develop into branches have been described, and it seems possible that injury in itself stimulates the further growth of the embryonic cells. Adventitious branches rarely develop from the upper nodes of whole 'leaves', and the best results are obtained by injuring the 'leaf'. Although slight injury, such as pricking the internodal cell, stimulates the formation of these branches, yet more severe injury, such as cutting off the 'leaflets' and terminal cell, results in the death of the 'leaf' before any branches appear.

The removal of branches, &c., such as has been described, besides injuring the plant, has a more important effect by upsetting the growth-correlations which exist in the plant. As long as the chief apices are actively growing and dividing, the further development of groups of embryonic cells which occur at the various nodes of the 'leaves' seems to be inhibited. The inhibition effect of growing apices on other embryonic cells is very clearly seen in *C. vulgaris*, where, at the base of the lateral branch, there are two dormant apices, which never develop unless the lateral branch is injured. Similarly, in *N. opaca*, the embryonic cells at the node of the 'leaf' seldom develop into adventitious branches and rhizoids unless isolated from the influence of the existing apices, and the greater the isolation from existing apices the more prolific the development of the adventitious outgrowths. For example, adventitious branches seldom develop from the

<sup>1</sup> Attempts were made to stimulate adventitious branch development from the upper nodes of the 'leaves' of *Chara vulgaris*, but no branches appeared. This is not surprising, since there are very few embryonic cells at these nodes.

basal nodes and never from the upper nodes of the 'leaves' of uninjured plants, but if whorls of 'leaves' are isolated and the lateral branches of the stem-node are cut off, then adventitious branches develop almost invariably from the basal nodes of most of the 'leaves', and quite often from the upper nodes as well. To obtain more certain development of adventitious branches at the upper nodes it is necessary to isolate them from the influence of the cells of the basal node, and this can be done either by injuring the internodal cell of the leaf, so that it soon dies, or by destroying the basal node. It seems, therefore, that existing apices and even other embryonic cells inhibit the development of the embryonic cells of the upper node of the 'leaf', but once their influence is removed development proceeds.

Although in most cases injury is necessary to stimulate the development of adventitious branches, yet its chief influence is probably not direct, but indirect, by disturbing growth-correlations.

Two types of adventitious branches occur, radial, i.e. like the main axis, and pro-embryonic.

Both types may grow from the same node, and even one type may grow from the basal node of the other, but there are indications of a seasonal distribution. The pro-embryonic type is dominant in winter, and in summer the majority of the branches are radial. This suggests that external conditions, probably by affecting the nutrition factors, have some influence in determining which type of branch develops. Besides resulting in a greater accumulation of stored food materials, the conditions in the summer months are much more favourable to growth, and may have a direct effect on the plant in determining which type of branch is formed. The effect of the more favourable conditions during the summer months is also seen in the number of lateral branches which develop at any one node of the main shoot. In winter there are seldom more than two, whereas in summer seven or eight are quite common.

In conclusion, I wish to express my thanks to Professor W. H. Lang for the help he has given me with this work. I am also indebted to Mr. J. Groves for his kind assistance in identifying various species of Characeae and in other ways.

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# The Evolution of the Tristichaceae and Podostemaceae. I.

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With Plate XIII.

INTRODUCTORY.

IN the present series of two or three papers I propose once more to deal with the two families of plants to whose study I have devoted much time during the last thirty years, and to consider further the question of their evolution, already touched upon in earlier publications (8, 11).

The general line of argument followed in this first paper is simple. I have already shown (11) that it is almost impossible to conceive of a gradual adaptation changing these plants from the condition of ordinary hydrophytes—of more or less quiet water, with a bottom of sand or mud—to their actual condition of water plants of swiftly running streams, usually with a bottom of smooth water-worn rock, but sometimes one of shingle; in either case a bottom into which the roots cannot penetrate. It would seem most probable that they are derived from land plants which grew upon the edges of waterfalls or rapid streams with stony bottoms; their flowers and fruits, indeed, are more obviously of 'land' types than those of most water plants. In another paper (9) I have shown that once in the water there are practically no differences of conditions to which these plants can be adapted; they live under conditions which allow of but little variation in food, light, temperature, &c., and only very rarely have they any competition with other forms of life. Even among themselves the competition is but slight. The action of natural selection is thus to all intents and purposes eliminated. The families, however, are characterized by the most extraordinary degree of differentiation, as a glance at the plate will show. Their general morphology exhibits especially such features as in former years were taken for granted as the signs of the highest possible

adaptation, though there is nothing to which to be adapted that is not common to all.

The characters employed in the distinction of the species, genera, and tribes are then put together in order for comparison and analysed; they number about a hundred or rather more, apart from differences of the fluctuating kind in length, breadth, thickness, &c. An attempt is made to indicate that about 85 per cent. of them are characters that could not in any case have been the subject of selection, that in about 97 per cent. the early stages, if they existed, could have been of no possible use, that in about 78 per cent. even the mature stages cannot be conceived of as useful, and that about 38 per cent. are not even capable of having intermediate stages in their development. To 35 per cent. of the total all these objections apply, and to 42 per cent. three of them, leaving only a bare 23 per cent. to which less than three of these objections are raised. Only three insignificant characters have none. The only reasonable probability, therefore, seems to be that most of the features that characterize the species and higher groups within the family were acquired at *single steps*, or (in a number of cases, it may be) as the result of a more or less predetermined or orthogenetic course of evolution, controlled by factors (probably internal) that we do not understand, which caused mutations to follow a certain course.

The object at which I am aiming in this paper is to show that it is at least highly probable that evolution proceeded by definite, and *often large steps*, so that a new species or even the first member of a genus, or larger group, might suddenly appear. It is not contended that the steps must always be large; on the contrary, it is much more probable that they are most frequently small, giving rise to varieties, forms, races, &c.; but at times a large step will occur, and will result in the formation of a new species, which will most probably, perhaps, be more or less sterile with its nearest relatives.

Such views are of course much opposed to those of the school of natural selection, as were the similar ones held by Mivart, Owen, and others sixty years ago, but it must not be forgotten that the idea of very gradual change came in only with the theory of natural selection, and becomes quite unnecessary again with the decay of that theory. It may be worth while to quote from Mivart, even now. On p. 5 (2) he says that the object of his book is to maintain the position 'that Natural Selection acts, and indeed must act, but that still, in order to account for the production of known kinds of animals and plants, it requires to be supplemented by the action of some other natural law or laws as yet undiscovered'. This is a very clear statement of the position for which we are now contending, when added to that on p. 24, where, in outlining the argument, he says, 'that there are grounds for thinking that specific differences may

be developed suddenly instead of gradually', and again, 'that the opinion that species have definite though very different limits to their variability is still tenable'. On p. 51, after discussing Darwin's ideas of correlation, he says, 'but the idea that the modification of any . . . part . . . of an *Echinus* carries with it the effect of producing elongated flexible triradiate snapping processes is, to say the very least, fully as obscure and mysterious as what is here contended for, viz. the efficient presence of an unknown internal natural law or laws conditioning the evolution of new specific forms from preceding ones, modified by the action of surrounding conditions, by natural selection, and by other controlling influences', whilst on p. 132 he says, 'not only does it appear that there are barriers which oppose change in certain directions, but that there are positive tendencies to development along certain special lines'. Quotations upon somewhat similar lines might be made from Owen (3) and from others.

The experimental school demand evidence of the actual occurrence of such changes as have been indicated. That they need not occur very often in nature I have shown in (12), p. 212, where I suggested one mutation in fifty years on any small space of the earth's surface as sufficient. Mr. Udney Yule has reduced this to more accurate figures (14, p. 84) of one in fifteen to one in thirty years. In any case, the chance of seeing such a thing is practically nil, and, if the result were subsequently found, there is no doubt that in the present stage of science it would be regarded as a relic. We are as yet distant from the period when we shall be able to produce mutations of this type at will, though there seems every probability that we shall at some future time be able to do so, and the work of Morgan and others already shows that nuclear changes may be accompanied by considerable morphological differences, as indeed one would expect when one remembers that characters are usually more numerous than chromosomes.

In view of this at present almost insuperable difficulty, the line that I have taken is to try to show that it is all but impossible for evolution to have taken place here in any other way than (often) by sudden and large mutations, unless in some cases by the result of some 'orthogenetic' force. The great bulk—indeed, practically all—of the characters cannot be explained in terms of advantage, so that selection in the ordinary sense could not produce them. And about two-fifths of them do not admit of intermediates between themselves and their most nearly related forms, whether directly between or round by way of a hypothetical ancestor combining the characters.

A distinguished botanist, who read over my manuscript about ten years ago, said that I had no right to suppose 'that the evolution of these rare and very peculiar families had followed lines the same as those of the rest of the flowering plants'. But until evidence is brought up that renders

adaptation, though there is nothing to which to be adapted that is not common to all.

The characters employed in the distinction of the species, genera, and tribes are then put together in order for comparison and analysed; they number about a hundred or rather more, apart from differences of the fluctuating kind in length, breadth, thickness, &c. An attempt is made to indicate that about 85 per cent. of them are characters that could not in any case have been the subject of selection, that in about 97 per cent. the early stages, if they existed, could have been of no possible use, that in about 78 per cent. even the mature stages cannot be conceived of as useful, and that about 38 per cent. are not even capable of having intermediate stages in their development. To 35 per cent. of the total all these objections apply, and to 42 per cent. three of them, leaving only a bare 23 per cent. to which less than three of these objections are raised. Only three insignificant characters have none. The only reasonable probability, therefore, seems to be that most of the features that characterize the species and higher groups within the family were acquired at *single steps*, or (in a number of cases, it may be) as the result of a more or less predetermined or orthogenetic course of evolution, controlled by factors (probably internal) that we do not understand, which caused mutations to follow a certain course.

The object at which I am aiming in this paper is to show that it is at least highly probable that evolution proceeded by definite, and *often large steps*, so that a new species or even the first member of a genus, or larger group, might suddenly appear. It is not contended that the steps must always be large; on the contrary, it is much more probable that they are most frequently small, giving rise to varieties, forms, races, &c.; but at times a large step will occur, and will result in the formation of a new species, which will most probably, perhaps, be more or less sterile with its nearest relatives.

Such views are of course much opposed to those of the school of natural selection, as were the similar ones held by Mivart, Owen, and others sixty years ago, but it must not be forgotten that the idea of very gradual change came in only with the theory of natural selection, and becomes quite unnecessary again with the decay of that theory. It may be worth while to quote from Mivart, even now. On p. 5 (2) he says that the object of his book is to maintain the position 'that Natural Selection acts, and indeed must act, but that still, in order to account for the production of known kinds of animals and plants, it requires to be supplemented by the action of some other natural law or laws as yet undiscovered'. This is a very clear statement of the position for which we are now contending, when added to that on p. 24, where, in outlining the argument, he says, 'that there are grounds for thinking that specific differences may



be developed suddenly instead of gradually', and again, 'that the opinion that species have definite though very different limits to their variability is still tenable'. On p. 51, after discussing Darwin's ideas of correlation, he says, 'but the idea that the modification of any . . . part . . . of an *Echinus* carries with it the effect of producing elongated flexible triradiate snapping processes is, to say the very least, fully as obscure and mysterious as what is here contended for, viz. the efficient presence of an unknown internal natural law or laws conditioning the evolution of new specific forms from preceding ones, modified by the action of surrounding conditions, by natural selection, and by other controlling influences', whilst on p. 132 he says, 'not only does it appear that there are barriers which oppose change in certain directions, but that there are positive tendencies to development along certain special lines'. Quotations upon somewhat similar lines might be made from Owen (3) and from others.

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A distinguished botanist, who read over my manuscript about ten years ago, said that I had no right to suppose 'that the evolution of these rare and very peculiar families had followed lines the same as those of the rest of the flowering plants'. But until evidence is brought up that renders

it in some degree probable that their evolution followed lines that are *essentially* different, it would seem better to assume, as has always been done so far, that the lines followed were the same here as in other cases though perhaps pushed to unusual extremes, or more affected than commonly by some of the factors which control evolution. These families are by no means rare in the few positions which are suitable to them, but occur there in abundance without any competitors. An analysis, such as is given below, shows that their characters are much the same in type as in other families, but with an unusual prevalence of dorsiventral features—probably due to an unusual degree of operation of the special factors to which dorsiventrality is due.

#### ARGUMENT.

In my paper (11) I have shown that it is in a very high degree unlikely that these plants could have owed their origin to the modification of other water plants of more common types, such as grow in quiet water, or upon muddy or sandy bottom, into which their roots can penetrate. A sudden change occurs when the bottom ceases to be thus penetrable, and becomes stony, so that the roots can no longer get a hold in the old way. One can hardly imagine the bottom changing gradually from a penetrable one to a rocky one, and I have suggested that it is much easier to imagine these plants developing from land plants which grew upon the edges of the rapid streams in which the Podostemaceae are found.

There are many points in favour of this view. These plants, for example, do not contain the large intercellular spaces that are characteristic of the water plants of quiet water, and it is simpler to suppose that such spaces never appeared in the ancestral tree than to suppose that they appeared, and then completely disappeared again. In the second place, their flowers are typical 'land plant' flowers, with more resemblance, perhaps, than is usual in water plants to the flowers that one sees in typically terrestrial families. They wait the fall of the water-level to expand, and then, when touched by the air, open above the water. Another and still stronger argument in favour of this view is to be derived from the way in which they produce seed. Most ordinary water plants produce small amounts of seed, but seed that comes to no harm should it happen to fall into the water. These plants, on the other hand, produce enormous amounts of seed (a character quite unusual in water plants), and any seed that is not firmly fastened to the rock, or caught in a crevice, will (if not already blown away, as is most probable) inevitably be lost and come to nothing when the water rises, for it will be washed away, and the chance of its catching again upon some portion of naked rock covered by rapidly moving water, where alone it can grow, is essentially nil. The early stage of these families must have been with many seed, for all members of

both families show this character, with the solitary exception of *Farmera*, a small genus with one species in Ceylon, and one in South Kanara. In this genus, instead of a great number of small seeds, there are two or four large ones, but no intermediates occur in any others of the family.

If a locality where these plants are growing be watched from year to year, it will be noticed that the number of individuals of any species remains practically constant, and when one realizes that a plant may set as many as 50,000 seed, it is clear that a vast amount of destruction goes on, a phenomenon which at first sight would appear to form a splendid basis for selection. One soon finds, however, that the destruction of *ungerminated seed* (by blowing or washing away) is so enormous that in place of one parent there may start perhaps two to ten seedlings, most of which are washed away before they have formed their first leaves. There is no crowding and competition among the seedlings, but the rock is usually so bare that it looks as if nothing were growing upon it. It is thus clear that the characters that show in the mature plant cannot in general be the subject of selection, for by the time that they appear the competition, such as it is, is practically over.

The only means of transport from one river to another is in general for the seeds to adhere to the feet of wading birds, but unless they arrived in great numbers (which is very improbable) the chance of selection producing suitable forms would be practically nil; yet they differ in general from river to river, though the conditions are often, in essence, absolutely identical.

The seeds are evidently little if at all modified from the 'land' type with which they began. If the families were really specially adapted to their mode of life, one would expect to find what in fact is conspicuous by its absence—some arrangement to fasten the seeds to the rock for germination, so that they should not be washed away. Yet this occurs in one species only, in *Farmera metzgerioides* in Ceylon (not in the other *Farmera*), the fruit remaining closed, holding the seeds against the rock. The difference between this species and the other *Farmera*—an indehiscent fruit—is valueless if not complete, and can hardly be supposed to have been acquired by degrees, or by selection, though, when once acquired, it would be so useful that the new species would not be likely to disappear again. The whole evidence derived from the fruit and seed, and their behaviour, goes to favour the idea of land ancestry, and to discount any supposition of selection.

In another paper (9) I have pointed out that the conditions under which these plants exist and thrive are perhaps the most uniform that occur in the flowering plants, rivalled only in this respect, if at all, by the conditions of the diatoms of the tropical seas, the Saprolegnias of tropical insects, or by those of one or two other groups of water plants or of insects in the tropics.

They grow as a rule upon a substratum of firm smooth water-worn rock, in rapidly running water; but one or two species of *Tristicha* and *Podostemon*, and perhaps others, grow upon a shingle bottom in rippling water. None are ever found in still water, nor upon a bottom of mud or sand. They never possess penetrating or absorbent roots, but only organs of attachment, which are closely appressed to the rock or stones.

They also grow under the most extremely uniform conditions of light and temperature, for they are all but confined to the tropics, and there to moderate elevations, whilst in the 'cold weather' they are only represented by seeds. The conditions of food-supply, as they get all their food from the rapidly moving water, are also essentially the same for all. They have little or no competition with other forms of life, for they share their habitats only with an occasional moss; ordinary water plants are quite incapable of life under such conditions as these families affect. There is not even much competition among themselves, owing to the enormous destruction of seed that has already been pointed out.

Thus in their mode of life there is no serious variety of conditions to which they have to be adapted. The large forms do not grow in very shallow water, but that is about all; and they could not in any case do so. Nothing can be seen which seems to be an adaptation to the different speeds of water current; some of those of very rapid water look but ill adapted to such an existence, while some of those of slowly moving water look very well suited to swift currents.

The action of natural selection is thus practically eliminated from the evolution of these families, since their common ancestor took to life under the conditions which they affect. Their evolution cannot have been in response to a necessity of adaptation to varied conditions, for they all live under conditions which are essentially the same, and have practically no competition, especially with other forms of life. It is probable that one or two species could quite well cover *all* the places where they grow; yet the families have developed into twenty or thirty well-marked genera, with perhaps a couple of hundred well-defined species, showing a much greater degree of difference than any other family of flowering plants, or perhaps even of thallophytes. It will suffice to give a few illustrations of this extraordinary range of form. The Plate in this article gives a few of the species of Ceylon and India only, and there is as much variation (and different again) in Africa and America.

Now though the differences among these plants are so well marked, and often so striking, the separation characters actually employed by the systematists, which cover all the serious features of difference, do not amount to so great a number as might perhaps be expected. They are given in the following enumeration grouped—whether they be of species, genus, or sub-family—under the heads of members concerned,

and if one leave out those which are mere differences in size they add up to rather more than 100.

Against each character, with three exceptions (F 6, 7, 14), are placed one or more of the letters S, E, L, I, whose significance is as follows :

- S. Cannot be supposed to be the subject of natural selection.
- E. The early stages could be of no conceivable value.
- L. The late or mature stages can scarcely be of possible value.
- I. Intermediate stages are not possible.

SE -- A	1	Hypocotyl growing erect	growing laterally
- E -- B	1	Primary axis large	small
SE - I	2	" " producing creeping roots	creeping shoots
SELI	3	" " floriferous	not floriferous
- E -- C	1	Creeping root thread-like	ribbon-like
- E --	2		goblet-like
SE - I	3	" " of one form	of two forms
SELI	D 1	Thallus from primary axis	from secondary
SELI	2	" of shoot nature	of root nature
- E --	3	" narrow	broad
SELI	4	" branched before shoots	behind shoots
SELI	5	" endogenously branched	exogenously
SE --	6	" ribbon-like	crustaceous
SEL -	7	" apex flabelliform	linear or ribbon-like
SELI	8	" of one form	two forms
- E --	9	" attached at base	all over lower surface
SELI	10	" attached by root-hairs	by haptera or feet
- E -- E	1	Secondary shoots long	short
- E --	2	" " normal	thalloid
- E --	3	" " floating	creeping
SE --	4	" " erect radial	dorsiventral
SEL -	5	" " narrow oblong, &c.	broad helmet-like
SELI	6	" " tetrastichous	distichous
SELI	7	" " 1-flowered	many-flowered
SEL -	8	" " on thallus margin	on upper surface
SELI	9	" " branched	unbranched
SELI	F 1	Leaves alternate	opposite
SELI	2	" in two ranks	in three ranks
SELI	3		in four ranks
SELI	4		in many ranks
SEL -	5	" equal	unequal
- - - -	6	" large	small and delicate
- - - -	7	" short	long
SE --	8	" smooth	with small papillae
SE -	9		with large excrescences
SELI	10	" monomorphic	dimorphic
SEL -	11	" amplexicaul	not so
SE --	12	" enlarging at base to bract	not so
SE --	13	" ovate	oblong, linear, &c.
- - - -	14	" simple	laciniate
SEL -	15	" bipinnatisect	multipinnatisect
SELI	G 1	Inflorescence racemose	fasciculate
SELI	2		of solitary flowers
SEL -	H 1	Spathe erect	prostrate
SEL -	2	" tubular	capsular
SELI	3	" bifid	opening on one side
SELI	4		" by several teeth
SELI	5		" irregularly

SEL - J 1	Bracts equal	dorsiventral
SEL I 2	„ monothecous	ditheous
SEL - K 1	Flowers terminal	on upper surface of thallus
SEL - 2	„ on long stalks	nearly sessile
SEL - 3	„ subtended by ordinary leaves	by larger leaves
SEL I 4	„ on terminal shoot	from dorsiventral cupule
SEL - 5	„ exserted	enclosed in spathe
SEL - 6	„ erect in spathe	curved over
SE - - 7	„ actinomorphic	zygomorphic
SEL I 8	„ 3-merous	2-merous
SEL I 9	„	polymerous
SE - - 10	„ with perianth	without
- E - - 11	„ anemophilous	entomophilous
- E - - 12	„ chasmogamic	cleistogamic
- E - - L 1	Perianth coloured	scarious
SEL - 2	„ equalling ovary	exceeding ovary
SEL - M 1	Stamens in whorl(s)	unilateral
SEL I 2	„ in one whorl	in two whorls
SEL I 3	„	in three whorls
SEL - 4	„ free	monadelphous
SEL I 5	„ three	two
SEL I 6	„	one
SEL I 7	„	many
- E I - 8	„ short	long
SEL - 9	„ straight	curved over ovary
SEL - 10	„ filament thread-like	petaloid
SEL I N 1	Staminodes two	three
SEL I 2	„	none
SEL - O 1	Anther loculi discrete	contiguous
SEL I 2	„ introrse	extrorse
SEL - 3	„ not contorted	contorted
SEL - 4	„ entire	emarginate
SEL I P 1	Pollen simple	twinned
SEL - Q 1	Ovary free	adnate to androecium
SEL - 2	„ sessile	stalked
SEL I 3	„ of three carpels	of two
SEL - 4	„ symmetrical	zygomorphic
SEL - 5	„	asymmetrical
SEL I 6	„ with one fertile loculus	with two
SEL I 7	„	with three
SEL - 8	„ ovate	obovate
SEL - 9	„	lanceolate, &c.
SEL - R 1	Style none	elongated
SEL - S 1	Stigma linear	rostriform
SEL - 2	„	plurifid
SEL - 3	„	membranous dilated
SEL - 4	„ lanceolate corniform	broadly cuneiform
SEL - 5	„ entire	toothed or lobed
SEL - 6	„ capitate	separate
SEL - 7	„ persistent	deciduous
- E L - 8	„ short	long
SEL - 9	„ free	partly united
SEL - T 1	Placenta in centre of ovary	displaced to one end
SEL - U 1	Ovules many	two
SEL - 2	„	four

SEL - V	1	Pedicel of capsule short	long
SEL I	2	" " ribbed	smooth
SEL - W	1	Capsule stalked	sessile
SEL I	2	" ribbed	smooth
SEL I	3	" 8-ribbed	12-ribbed
SEL -	4	" ribs equal	unequal
SEL -	5	" valves equal	unequal
SEL I	6	" valves both remaining	one valve dropping
SE - I	7	" dehiscent	indehiscent
SEL I	8	" bilocular	unilocular
SEL I	9	" with persistent stigma	deciduous stigma
SEL -	10	" erect	nodding
SEL -	11	" round	flat
- E I - X	1	Seeds small	large
SEL -	2	" many	few
SEL - Y	1	Cotyledons opposite	at angle of 135°

It is impossible to discuss these characters one by one, but very little consideration is required in most cases to see that in general there seems good reason for placing the signs against those characters where they are given. In the case of the first (S), for example, it is absurd to imagine natural selection deciding (A 1) that the hypocotyl shall grow laterally rather than erect (or vice versa), (D 1) that the thallus shall be from a primary rather than a secondary axis (or vice versa), (K 8) that the flower shall be 2-merous rather than 3-merous (or vice versa), or (W 2) the capsule ribbed rather than smooth (or vice versa); and the same in many more cases. In view of the fact that we have shown above that natural selection has no foothold from which to operate in these families, and that 102 out of 119 of these characters, or 85 per cent., could not, it seems to me, have been in any case the subject of its work, I think that it is fairly safe to rule it out as being of no serious importance in the evolution of these families from their first members to their present condition of considerable differentiation. And if there is no selection, there seems no reason for gradual acquisition of characters.

The second objection (E), that the early stages of a character could be of no conceivable use, applies to very nearly all, in fact to 116 out of 119, or to 97 per cent. The only remaining ones are F 6 (leaves large or small and delicate), F 7 (leaves short or long), F 14 (leaves simple or lacinate), and possibly F 5 (leaves equal or unequal). Even in these it is hard to think of early stages as being of any use. Use is ruled out as a serious factor in the evolution. Nothing can survive that is seriously *dis*-advantageous, but the majority of the morphological characters must be looked upon as being neither the one thing nor the other.

But if early stages are useless, then, unless there be some orthogenetic factor at work, it is hard to conceive any reason why they should ever need to exist, and the probabilities in favour of sudden origin are much strengthened. Natural selection seems completely ruled out. *Every plant*

*and every character must pass through the sieve of natural selection before it can survive at all*, and will be killed out if unsuitable, but that is as far as natural selection goes. It tends to *preserve* the type by killing out unsuitable or disadvantageous abnormalities, but does not lead to the production of new forms.

Not only are early stages of most of the characters useless, but in 93 cases out of the 119, or in 78 per cent., we find the mark L, implying that no use can be found for the mature stages. Here, again, there is no need to labour the point, when once the characters are set down as above. What use (as against the other, or against an intermediate, when such is possible) can be conceived for either one of such pairs as D 1 (thallus from primary *or* secondary axis), F 1 (leaves alternate *or* opposite), G 1 (inflorescence racemose *or* fasciculate), M 1 (stamens in a whorl *or* unilateral), N 1 (staminodes two *or* three), Q 1 (ovary free *or* adnate to androeceum), S 1 (stigma linear *or* rostriform), or W 1 (capsule stalked *or* sessile), to take only the first in each of a few sections?

Finally, in quite a large number of cases (45 out of 119, or 38 per cent.), intermediate characters, whether directly between, or by way of imaginary ancestors, are not even possible. This is obvious in such cases as B 2 (primary axis producing creeping roots *or* shoots), D 4 (thallus branched behind *or* before the shoots), D 5 (thallus branched endo- *or* exogenously), F 1 (leaves alternate *or* opposite), J 2 (bracts mono- *or* dithealous), K 8 (flower 3- *or* 2-merous), M 2 (stamens in one *or* two whorls), Q 6 (ovary with one *or* two loculi), W 2 (capsule ribbed *or* smooth), &c., &c. If intermediates are not possible, then the characters must have been acquired in single steps. There are many more, unmarked, in which intermediates are all but impossible or inconceivable.

Usefulness, and selection for usefulness, may thus, it seems to me, be left completely out of account in the differentiation of these forms, and if it is so often impossible for intermediates to occur, there seems little reason for refusing to consider seriously the view that evolution of most of the characters was by single steps, which produced one, or more probably several, of them at once, and thus gave rise to new species or even to individuals of higher rank. As I have several times remarked, one would expect any serious nuclear change to imply a good deal of differentiation.

That such an analysis of characters of differentiation is not exceptional may be seen by taking any other group of organisms, and analysing the characters in the same way. As an illustration we have taken the first group (the African *Crotalaria*s) that came to hand in looking over the Journal of the Linnean Society (1).



S E - I	Plant	annual	perennial
- E - -	"	herb	shrub, tree
S E - I	"	decumbent	erect
S E - I	"	branched	not
- E - -	"	glabrous	not
S E - I	Stem	herbaceous	woody
S E - I	"	erect	pro- or decumbent
- E - -	"	4-25 cm.	25-70 cm.
S E L -	"	winged or angled	not
S E L I	"	2-winged	4-winged
- E - -	"	glabrous	hairy
- E L I	"	hairs yellowish	whitish
S E L I	Leaf	simple	3-foliolate
S E L I	"	"	4-5-foliolate
S E L I	"	all 3-foliolate	lower so, upper simple
S E L I	"	"	upper so, lower simple
S E L -	"	sessile	petiolate
- E L -	"	petiole shortish	longish
- E - -	"	filiform	linear
- E - -	"	lanceolate (or ob-)	elliptical
- E - -	"	ovate (or ob-)	oblong
- E - -	"	"	rotundate, &c.
- E L -	"	acute	obtuse
S E L -	Petiole	present	absent
S E L -	"	7-12 mm.	shorter or longer
S E L I	Stipules	present	absent
- E - -	"	foliaceous	small
S E L -	"	sessile	petiolulate
S E L -	"	lanceolate	semilunate
S E L -	"	obcordate	other shape
S E L -	"	equally cordate below	unequally
- E - -	Leaflets	elliptic, oblong, ovate	cuneate, lanceolate, &c.
S E L -	"	sessile	stalked
S E L -	"	intermediates long	not so
- E - -	"	5-15 mm. long	15-25 mm.
- E - -	"	glabrous	hairy
- E L -	"	pubescent	tomentose
- E L -	"	hairy below	not so
S E L I	"	granulose-punctate	not so
- - - -	"	coriaceous	not so
- - - -	"	fleshy	not so
S E L -	"	emarginate	not so
S E L I	"	blackening when dry	not so
S E L I	Peduncle	1-2-flowered	3-6 or more
- E L -	Bracts	cordate-ovate	not so
- E L -	Bracteoles	ovate, oblong, linear	lanceolate, &c.
- E L -	"	narrow	broad
- E L I	Flowers	in racemes	solitary
S E L I	"	"	in heads
S E L I	"	"	in panicles
S E L I	"	"	in spikes
S E L -	"	crowded	loose
S E L -	"	long-stalked	short-stalked
S E L I	"	twinned	not so

- E - -	Flowers	small or minute	large
S E L -	"	near leaves	distant
S E L -	Calyx	4-5 mm. long	of other length
S E L -	"	lobes broad	lanceolate
S E L -	"	one lobe foliaceous	not so
S E L -	"	pilose	pubescent
- E L -	Wings	long	short
- E - -	Petals	yellow, lined, &c.	not so
S E L -	Standard	longer than rest	shorter
- E - -	Keel	5-9 mm.	10-20 mm.
S E L -	"	beaked	not so
S E L -	"	long-beaked	short
S E L -	"	naviculariform	uncinate
S E L -	"	dorsally rotundate	right-angled
S E L -	"	"	semi-orbicular
S E L -	"	genuflexed	not so
S E L -	"	with bent beak	with straight
S E L -	"	self-coloured	lined with colour
S E - I	"	purple or violet	yellow
S E L -	"	falcate	not so
S E L -	Pod	stalked	sessile
S E L -	"	stalk 16-20 mm.	25-30 mm.
S E L -	"	with few seeds	many
S E L I	"	with 1-2 seeds	with 3 seeds
S E L -	"	2-4 mm. long	longer, to 30 mm.
S E L -	"	globose, cylindrical	oblong, oviform, &c.
S E L -	"	angulate	rounded
S E L -	"	rugose	not so
S E L -	"	subglaucescent	not so
S E L -	"	glabrous	hairy within
S E L -	"	hirsute	villous
S E L I	"	with brown hairs	with white

A comparison with the characters of the Podostemaccae and Tristichaceae, already given, shows the following result :

	<i>Pod. and Trist.</i>	<i>Crotal.</i>
S. Selection not possible	%	%
E. Early stages useless	85	68
L. Late stages useless	97	98
I. Intermediates impossible	78	73
	38	26

In both cases a very large proportion of the characters is subject to these objections. Proportionately more floral characters are employed in the case of the two families that we are chiefly considering (78 to 41 vegetative, against 43 to 43), and in these the objections are more pronounced, as one would expect. The objection S, for example, applies to 73 per cent. of the vegetative and 92 per cent. of the floral characters of the families, and to 56 per cent. and 81 per cent. in *Crotalaria*; and the other objections show similar results.

It is clear that natural selection and usefulness must in general be

ruled out as serious factors in the evolution of the numerous genera and species of these families from their common ancestor or ancestors, and that a large proportion, if not the bulk, of their characters must be indifferent. This is not to be interpreted as meaning that there is no adaptation in the families, but the adaptation is chiefly a family, and not a specific one. This, by the way, as de Vries has pointed out (12, p. 224), is a very general rule with regard to adaptation. It is very rarely simply specific, and is much more commonly generic or family, as if, once a species with good adaptation to something or another had passed through the sieve of natural selection, it tended to give rise with some rapidity to a number of descendants, all inheriting the same adaptation.

The first origin of these families was due, one may imagine (11), to the appearance of a plant which showed the necessary adaptations to their very peculiar mode of life, but once this had appeared, and had passed through the sieve of natural selection, the same adaptations appeared in all the members of the families which descended from the original ancestor or ancestors—all of them can live on smooth rocks in rapidly moving water, producing their flowers above the water-level in the drier weather. And all, it may also be noted, with the one solitary exception of *Farmera metzgerioides* in Ceylon, show the same lack of an adaptation for holding their seeds to the rock for germination—an adaptation which one would rather have expected to find at the very beginning of the families.

There is, however, one very striking case of a *progressive* adaptation in these families, to which we shall devote more attention in later papers, but which, so far as one can see, has no element of serious usefulness attached to it, but is simply what one may perhaps call a 'compulsory' adaptation (cf. 8, pp. 438-9) to a force which is always acting upon all their members. As their roots cannot penetrate the substratum, the plants are always deprived of one-half of their polarity, and are always, no matter how they may try to grow, subject to the maximum influence of plagiotropism. In correlation with this, one finds that they show, as one passes from the widely distributed—and therefore probably old—members of the families, to the narrowly distributed—and therefore probably young—members, an increasing dorsiventrality of structure, which in the most modified members reaches probably the highest point shown in any flowering plants, and one hardly equalled by any cryptogams.

Not only do a great proportion of the characters given, both for the two families and for *Crotalaria*, show the single objections, of which we have already spoken, but most of them show two or more at the same time.

In both cases about three-quarters (79 per cent. and 71 per cent.) show three or four of the four possible objections (always E, usually S and L, and often I), about one-quarter show two or one, and those with none are negligible.

The actual figures are :

	<i>Pod. and Trist.</i>		<i>Crotal.</i>	
	%		%	
S E L I	42	35	15	17
S E L -	48	40	38	44
S E - I	3	2	6	7
- E L I	—	—	2	3
Three or four objections	93	77	61	71
S E - -	9	7	—	—
- E L -	3	2	8	9
Two objections	12	9	8	9
- E - - }				
One objection }	11	9	15	17
No objections	3	2	2	3
Total	119		86	

The only conclusion that one can come to about the development of the actual characters, that seems to me reasonable, is that in many, if not even in most cases, they have been acquired in single steps. No other conclusion is possible in regard to those marked I, such as D 2 (thallus of shoot *or* root nature) or F 1 (leaves alternate *or* opposite) or K 8 (flowers 3-merous *or* 2-merous). Almost two-fifths of the characters come under this description, and in the case of many more it is at least most probable that no intermediates ever existed.

Such a conclusion seems repugnant to many biologists, but their dislike is probably due to the lingering influence of the theory of natural selection. It must never be forgotten that this theory struck out what was then a new line in biological science, and one quite contrary to that which had always been previously held—that species had been created in more or less the exact form in which they most commonly occur. Two generations have grown up under the influence of the teaching introduced by the theory of natural selection, that change is necessarily minute and gradual. But such changes can hardly occur unless one accept natural selection (and usefulness at every stage) as a guiding agent, and now that it is seen to be of so little value in this respect—at any rate, as regards differences within a genus or family—the chief reason for the acceptance of very gradual change has disappeared.

In such cases as that we are considering, where selection for usefulness is to all intents and purposes out of court, gradual change is only possible if there be some 'orthogenetic' force at work. There seems to me, however, no necessity to assume that such a force must produce its results only by very gradual stages, and not by steps which are often sufficient to mark new species at once. If the orthogenetic changes were very gradual, one would expect that the new species would very often occupy large areas, for one would expect, just as in the case of natural selection, that the same change would go on over large numbers of the parent species.

It would seem simpler, especially in view of the work done by Mr.

Udny Yule and myself (14, 13) which goes to show that there is a mathematical relation between the numbers of species in genera, to adopt for the present the hypothesis that changes may occur that are sufficiently large to produce new species at single steps, and to see to what that hypothesis may lead us.

The point for which we are contending will be brought more clearly forward if we consider one or two actual instances among the plants of these two families. Take, for example, the case of the Tristichaceae. This family (10) consists of three genera only—*Tristicha* in tropical Asia, Africa, and America, *Weddellina* in Guiana and North Brazil, and *Lawia* in Ceylon and southern India. Now while *Weddellina* is in general of the same morphological type of construction as *Tristicha*, differing chiefly in its bicarpellary ovary and many stamens, *Lawia* is an altogether distinct type, which does not allow of intermediates to link it up to the other two, nor to any members of the other family Podostemaceae.

The germination of the Tristichas has not been observed, but as the mature plants have—like the Podostemaceae—creeping roots giving rise to secondary shoots, it is probable that the germination is the same, an adventitious root arising from the hypocotyl, creeping over the rocks, and giving off secondary shoots. These shoots are in no way dorsiventral in *T. ramosissima* (India) (cf. Plate), and only slightly so in other species; but in a new species which I discovered near to Rio de Janeiro they are flat shoots with leaves on the upper side (like *Lawia*), but they have no endogenous shoots springing from them (as in that genus), whilst they bear the characteristic ramuli or short shoots of *Tristicha* at the edges. This form, therefore, cannot be regarded as in any way an intermediate between the non-dorsiventral Tristichas and *Lawia*, where the thallus is from the primary axis. In germination this latter gives rise to a short hypocotyl, which develops a few leaves at the upper end, and then grows out horizontally and laterally into the flat creeping thallus, which upon the upper side bears leaves directly, as well as the endogenous secondary shoots, appearing as tufts of leaves. To arrive at the construction of *Lawia*, which is quite unique, a very large step must be taken. A fusion of the thalli of the new (Rio) species of *Tristicha* would not begin to do it, for they are of secondary-axis type, and even then the ramuli have to be replaced by endogenous shoots or surface leaves. And in any case we can imagine no selection coming in, for one can see no advantage of one form against the other, nor, as we have seen, is there any difference in conditions that could call selection into operation. The cupule of *Lawia* is also a large step removed from the several free bract-like leaves subtending the flower of *Tristicha*.

While not, perhaps, so large as the steps called for to pass from *Tristicha* to *Lawia* (or from a common ancestor to both), those needed to

pass from *Tristicha* (or *Lawia*, if such were conceivable with the existing geographical distribution) to *Weddellina* (or from a common ancestor to both, or to all three) are also very large, for the number of carpels is reduced from three to two—only conceivable as one step—and the number of stamens is increased from a maximum of three to six or more (up to twelve or possibly more).

Or again, passing to the family Podostemaceae, take the case of the genus *Hydrobryum*. The genus is divided into two sections, one with an isolobous fruit having twelve ribs, the other with an anisolobous fruit having either eight ribs or none. Obviously there must have been large mutations; one cannot imagine fruits with nine, ten, and eleven ribs forming intermediate stages, nor any action of selection which should afterwards destroy them. The mutation, too, which should give rise to these would probably (as being unsymmetrical) be a really larger one than that which went direct from twelve to eight or vice versa. Nor can one imagine gradual changes from isoloby to anisoloby, or from the ribbed to the smooth fruits in the second section of the genus. Or take the thallus in this genus—some have a ribbon-like thallus branched in acropetal succession, some have a flat lichen-like thallus, more or less circular in outline, with a continuous growing edge in place of the growing-point of the first type (cf. Plate). These differences could not have come by gradual stages, especially when one remembers that one type of thallus presents no advantages over the other, and that there are no differing conditions to call out the action of selection.

Take, again, the genus *Farmcra*. Considerable mutations must have occurred to reduce the number of seeds from very many to very few (two to four), to place the point of origin of the secondary shoots behind instead of before the branches of the thallus, to cause the difference between the two species in that one has a dehiscent, the other an indehiscent, fruit, or to place the cotyledons at an angle of  $135^\circ$  and so make even the embryo dorsiventral.

#### SUMMARY.

The introduction gives a summary of the argument, which may be put in another way here. The following conclusions may perhaps be drawn:

(a) Owing to the uniformity of the conditions under which the Podostemaceae and Tristichaceae live (and one may say, with practical certainty, must always have lived) there is no foothold for any serious action of natural selection, once their primitive ancestor had passed through its action and had been allowed to survive.

(b) About 85 per cent. of their characters could not in any case have been the subject of natural selection.

(c) The early stages of about 97 per cent. of their characters could have had no conceivable use, and therefore there seems little reason why they should ever have existed in place of the mature stages.

(d) Even the late stage in about four-fifths is useless, and may perhaps be regarded as modification *de luxe*.

(e) Selection for the sake of advantage could therefore not have been of any serious importance, especially in view of the enormous destruction of ungerminated seed.

(f) The species are on the whole so well differentiated that a vast destruction of intermediates must have gone on if the characters were gradually produced, and this is all but inconceivable in view of (e).

(g) In about 38 per cent. of their characters intermediates are not possible, whilst in about 50 per cent. more they must be looked upon as highly improbable.

(h) The idea of gradual change only came in with the theory of natural selection, and is quite unnecessary except in connexion with that theory, which we have seen to be incapable of application in this case.

(i) There is no reason why alteration *must* be minutely graduated, and if one considers that changes analogous to chemical changes are at the bottom of most alteration, one will not expect such.

(j) It is probable, therefore, that in at least a large proportion of the species of these families the changes were large enough to create new species, or even larger groups, at once.

(k) Specific illustrations are given from both families, showing, for example, how impossible it is to imagine the genus *Lawia* (in Tristichaceae) arising by any but 'large' mutations.

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### DESCRIPTION OF PLATE XIII.

Illustrating Dr. J. C. Willis's paper on the Evolution of the Tristichaceae and Podostemaceae.

The figures are reproduced by photography from Plates V, XII, XV, XXVI, XXIX, XXXI, and XXXV of the author's paper 'Studies in the Morphology and Ecology of the Podostemaceae of Ceylon and India' in Ann. R.B.G., Peradeniya, vol. i, p. 267, 1902.

Fig. 1. *Tristicha ramosissima* (Wight) Willis.

Tristichaceae.  $1/7$  natural size.

Western Ghats of India, S. Kanara to Travancore.

Submerged plant from S. Kanara, floated out upon paper by Dr. C. A. Barber, and dried.

An almost mature plant in the vegetative condition before formation of the flowers. The nearest in type of construction to an ordinary water plant to be found in these families. Running right and left across the middle is the creeping thallus, in this case a thread-like root (probably adventitious from the primary axis), and upon it are borne, often in pairs, the secondary shoots with swollen bases (haptera) forming closely adherent attachments to the rock. These shoots bear branches of two kinds, (a) ramuli or short shoots (best seen to the far left) of limited growth with delicate moss-like leaves of one cell in thickness, and (b) ordinary branches that repeat the structure of the main stem. Later the basal portions of all the shoots produce flowers in numbers on short branches.

Fig. 2. *Lawia zeylanica*, Tul.

Tristichaceae.  $1/5$  natural size.

Western Ghats, Bombay to Travancore; Ceylon.

Submerged plants of var. *Gardneriana* (Ceylon only), in young vegetative condition (gathered in August), preserved in alcohol.

This genus is highly dorsiventral in the vegetative organs, but not in the flower. The figure shows two plants, about half-grown, upon a piece of rock which was chipped away from the rocky river bed. Each is composed of a flat thallus, in this case of stem nature, radiating from the point where the seed germinated, branching freely, and closely attached to the rock. With a lens there can be seen (especially in the middle on the right) whitish lines between the lobes, which are the marginal leaves of the lobes closely crowded together; and on the upper surface can be seen a suggestion of the leaves that are borne there. In some places endogenous secondary shoots, looking somewhat like volcanic craters, may be noticed. The flowers spring from these at a later period. Cf. p. 306 in Ann. Perad., l. c.

Fig. 3. *Podostemon subulatus*, Gardn.

Podostemaceae.  $1/5$  natural size.

S. India, Ceylon.

Submerged plants of var. *Mavoiiae* (Ceylon only), of same age as last, preserved in alcohol.

Plants of bilateral symmetry in the vegetative condition, growing in dense masses. On the left, detached, may be seen a portion of thallus, here a creeping thread-like root, attached to the rock, from which spring, endogenously as usual, and often in pairs, the secondary shoots, which grow more



or less vertically upward into the water. They bear leaves in two ranks, with a slight hollowing of the shoot on the upper side, and later bear the flowers, usually on lateral branches from the lower axils of the ditheous leaves. The flowers are very zygomorphic. Cf. p. 327, l. c.

Fig. 4. *Griffithella Hookeriana*, Wmg.

W. Ghats, Bombay to S. Kanara. 1/6 natural size.

Podostemaceae.

Submerged plants, taken at end of growing season, preserved in alcohol. The left-hand six from S. Kanara, with flowers ready to open, the right-hand six from Atgaon near Poona, with fruit already ripe.

Showing the extraordinary variety of form that may be assumed by the thallus, which is here of root nature. Besides these forms, which are only attached to the rock at the centre, there are others somewhat like *Hydrobryum lichenoides* below. The forms in the top left-hand corner are perhaps the most interesting; the root, which is the only vegetative body the plant possesses, forms a kind of goblet, attached only at the base. Only at the end of the season do the flowers appear on the little secondary shoots along the margins. Cf. p. 364, l. c.

Fig. 5. *Willisia selaginoides*, Wmg.

Podostemaceae. 1/6 natural size.

S. India, Anamalai Mts.; Burma (?).

Fruiting specimens, attached to the rock, taken in the dry season; the vegetative portions have fallen away.

The figure shows the basal portions of the secondary shoots in the fully mature condition with ripe fruits. The thallus is of root nature, crustaceous, and closely attached to the rock. The secondary shoots probably have long leaves in the vegetative condition, but this could not be made out, as the plant was only found at the fruiting stage. There are four ranks of scale leaves on each shoot, and a terminal sessile flower. Often the leaves and the cortex fall away, thus leaving the fruit on a pseudo-stalk composed of the central vascular tissues. Cf. p. 369, l. c.

Fig. 6. *Hydrobryum lichenoides*, Kurz.

Podostemaceae. 1/6 natural size.

Burma; Assam; W. Ghats, Bombay to Travancore; Ceylon.

Fruiting specimens, attached to rock, taken in dry season; the vegetative portions have fallen away.

Creeping on the rock is the thallus, which is of root nature, fairly regularly branched, and bearing secondary (endogenous) shoots at every bay. The leaves of these have fallen, but the fruits can be seen in the central upper piece of rock, which shows the thallus edgewise.

Fig. 7. *Hydrobryum olivaceum*, Tul.

Podostemaceae. 1/5 natural size.

Submerged plant, taken at the same time as 2 and 3, of var. *zeylanicum* (Ceylon only), preserved in alcohol, but first exposed to air for some time, to cause the fall of most of the dense mass of leaves with which it was covered.

The thallus, here of root nature, and green or olive-brown, is more or less rounded, is closely attached to the rock below, and bears endogenous secondary shoots. The growing-point is more or less continuous round the outer margin. The secondary shoots consist each of a mass of thread-like leaves, with no obvious growing stem before the flowering period; most of the leaves have been allowed to fall, to display the structure better. Towards the middle of the lower side may be seen the primary axis, an elongated hypocotyl crowned by a tuft of leaves. From this the thallus proceeds by lateral adventitious growth at the base of the hypocotyl, sometimes endogenous, sometimes exogenous. Cf. p. 379, l. c.





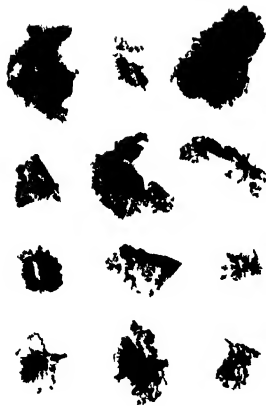
ESTERIA MONTANA



STYLARIA



STYLARIA



STYLARIA



WILLISIA SELAGINACEAE



WILLISIA

WILLIS-TRISTICHACEAE & PODOSTEMACEAE



WILLISIA

Hutch coll



# An Anatomical Study of the Variation in the Transition Phenomena in the Seedling of *Althaea rosea*.

BY

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With forty-two Figures in the Text

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## INTRODUCTION.

IN previous studies of polycotyly the forms examined in detail have been for the most part species in which the normal seedling possessed diarch symmetry. The discovery of a fairly large number of polycotylous individuals among the seedlings of a garden variety of *Althaea rosea* suggested the possibility of investigating the phenomenon of polycotyly in a form which generally exhibits tetrarch symmetry.

The anatomy of the normal seedling of *Althaea rosea* has been described in some detail by Gerard (8), and Dr. Thomas in her review of the seedling anatomy of Ranales, Rhoeadales, and Rosales (17), makes a number of references to *Althaea rosea* as an example of the 'Anemarrhena' type, and also figures several stages in transition. The present investigation was barely begun, however, when it became evident that, although many specimens conform to the type of structure described by these authors, the dicotylous seedling may exhibit considerable variety in its transition phenomena, and the need for a preliminary detailed study of these variations was clearly indicated. Accordingly a large number of

<sup>1</sup> This section is to be regarded as Part VI of the series of papers, 'Observations on the Anatomy of Teratological Seedlings', appearing in this Journal, 1918, 1920, 1921, 1925.

dicotylous seedlings have been examined—154 in all, of which 112 are perfectly normal in structure, whilst the remaining 42 all display some deviation from tetrarch symmetry.

The variability observed is evidently not a peculiarity of one special strain, as similar phenomena have been found in each of four strains examined.

#### THE STRUCTURE OF DICOTYLOUS SEEDLINGS.

##### *The normal seedling.*

The structure of the normal seedling with a tetrarch root will be briefly described for comparison with the variants to be mentioned later.

At the base of the cotyledon lamina three bundles are present, a median one and two laterals, these usually fusing in the upper portion of the petiole to form one kidney-shaped compound strand. The union is not, however, always so close, since in some seedlings one or both lateral strands may be quite distinct from the main bundle even at the base of the petiole.

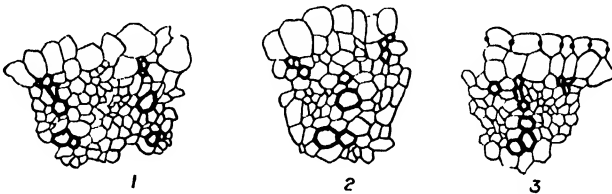
The protoxylem of the cotyledonary bundle becomes exarch at or immediately above the cotyledonary node, the phloem meanwhile undergoing division in the median plane. The lateral portions of the xylem now become separated as distinct groups which at first lie within the outer portions of the phloem masses, but later gradually approach the intercotyledonary plane, where they fuse in pairs to form two of the four root poles. During the passage towards the intercotyledonary plane the lateral xylem groups in some seedlings consist of a few elements irregularly arranged, whilst in others the constituent elements form a long file which assumes radial orientation. The latter condition was described by Gérard (8) as characteristic of the species.

In the majority of the seedlings examined the cotyledons were as yet unexpanded or were in the early stages of separation. At this age there is usually one well-marked epicotyledonary leaf, and sometimes the small rudiment of a second. At the cotyledonary node a slight asymmetry is frequently noticeable, the distance between the cotyledon bundles being somewhat greater on the side of the first epicotyledonary leaf than on the other. In a normal seedling this does not result in any appreciable delay in the formation of the intercotyledonary root pole: plumular traces are present in the upper end of the hypocotyl. In the majority of the seedlings these are in the form of desmogen or phloem strands, xylem elements being differentiated in only a small percentage of cases. The strands invariably remain median in position throughout their course and usually diminish rapidly in magnitude.

The tetrarch root possesses a well-marked pith.

*Seedlings showing variation from tetrarch symmetry.*

*A. Transient pentarchy.* In some seedlings the lateral xylem strands which usually unite in pairs in the intercotyledonary plane behave abnormally. Whilst on one side of the axis the xylem strands unite in the normal manner, on the other they remain apart for a short time, being separated by a strand of phloem. The axis at this point therefore shows some approach to pentarch structure, this being the more marked as the xylem strands in question assume the form of radial files of elements with exarch protoxylem. The change to the normal tetrarch structure may be accomplished in two ways. In some cases the median phloem strand slowly disappears and the two xylem groups gradually approximate, ultimately fusing to form one root pole. In other seedlings, as the median phloem group disappears, a central file of xylem elements gradually



FIGS. 1-3. Drawings showing three stages in formation of a root pole in the intercotyledonary plane in a seedling having a 'transient pentarch' phase.

develops, and with this the inner portions of the two lateral groups become associated, whilst the outer (protoxylem) elements persist in an isolated position on either side of the fully organized pole (Figs. 1-3). These isolated elements may ultimately disappear or may gradually approach the main xylem mass and become merged in it, both types of behaviour being sometimes shown by the elements associated with a single pole.

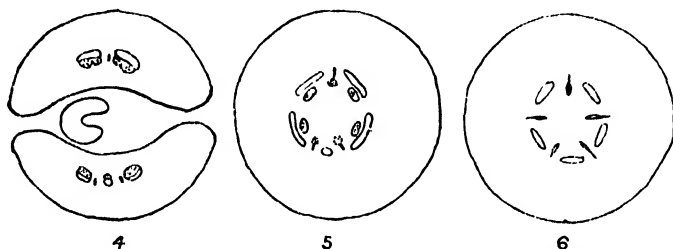
In some seedlings the delay in formation of the intercotyledonary root pole occurs on the same side of the axis as the first epicotyledonary leaf, the median phloem strand being then continuous with the plumular trace. In others, the abnormality occurs on the opposite side of the axis and the phloem group then appears only in the hypocotyl.

The phenomena described above have been observed in fourteen seedlings, the two methods of attaining tetrarch symmetry being about equally common. In two other seedlings both intercotyledonary poles are organized rather slowly, and the isolated lateral elements persist on either side of each pole for a time.

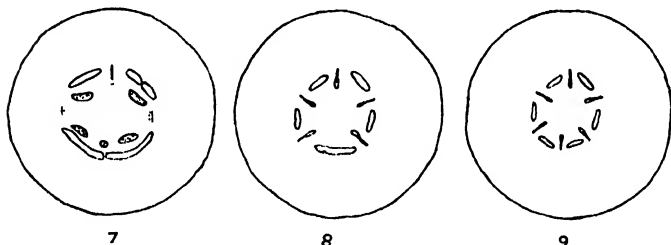
Another peculiarity which may be conveniently mentioned at this point is an unequal development of the lateral strands which constitute a pair. In one seedling, although tetrarch symmetry is attained, the two laterals forming one pole in the intercotyledonary plane contribute most

unequally to its xylem mass, whilst in a second seedling one of the poles of the intercotyledonary plane is formed by a single lateral, the corresponding lateral strand of the other cotyledon remaining in its original position near the cotyledonary plane and gradually dying out. In both seedlings the other pole is formed quite normally by the fusion of two equal lateral strands.

*B. Persistent pentarchy.* More or less persistent pentarchy has been found in twenty-three of the seedlings examined, but this structure may be produced in different ways.



FIGS. 4-6. Diagrams illustrating transition in a dicotylous seedling in which pentarchy results apparently from bifurcation of one median cotyledonary strand.



FIGS. 7-9. Diagrams showing transition in a dicotylous seedling with pentarch-hexarch structure: each lateral strand forms an independent pole; one pole in the cotyledonary plane appears only at a low level.

(i) In ten seedlings the lateral xylem strands on one side of the axis, instead of approaching one another, abruptly assume a radial arrangement and form two distinct poles, whilst on the other side a single pole is formed. This pentarch structure may persist throughout the seedling, or reduction to tetrarchy by fusion of poles may occur at a lower level.

(ii) In nine seedlings the transition is quite normal, and in the upper portion of the hypocotyl tetrarchy obtains. At a lower level in the hypocotyl, however, a fifth pole arises in the diagonal plane, sometimes the protoxylem and sometimes the metaxylem being the first to appear. In a few seedlings pentarchy of this type is associated with a transient pentarch phase, produced by the temporary independence of the laterals in the upper portion of the hypocotyl, the two being separated by a long or short tetrarch phase. It may be noted that in such cases the temporary inde-



pendence of the lateral strands occurs on the side of the axis opposite to that on which the fifth pole ultimately appears.

(iii) In one seedling, although both cotyledons are quite normal in form and venation, the kidney-shaped bundle of one cotyledon behaves quite abnormally in transition, since it develops two groups of exarch protoxylem and the phloem divides into three portions. The two lateral groups of xylem become separated as usual and fuse with the laterals of the other cotyledon to form two poles in the intercotyledonary plane (Figs. 4-6). Pentarchy in this seedling is produced apparently, therefore, by the bifurcation of one median cotyledonary strand.

(iv) Pentarch symmetry may be attained by still another method of which only one example has been met with. The vascular strand of one cotyledon behaves normally while the other strand is peculiar in that the phloem fails to divide in the cotyledonary plane, and in place of the median protoxylem there are a few metaxylem elements (Fig. 7). In the hypocotyl the small median xylem group disappears, while each of the associated lateral xylems forms a pole. The other three poles of the pentarch hypocotyl are formed from the vascular strand of the other cotyledon, the median and each lateral xylem group giving rise to a pole (Fig. 8). At a lower level in the hypocotyl hexarch structure is attained as the result of the appearance of a xylem pole in the cotyledonary plane (Fig. 9).

(v) In one seedling, after normal transition resulting in the production of tetrarch structure, pentarchy is produced by the bifurcation of one of the poles in the intercotyledonary plane.

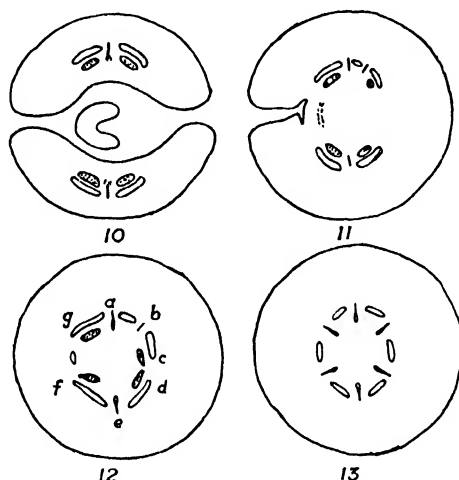
*C. Hexarchy.* Hexarch structure is comparatively rare, having been found in seven seedlings only. Three distinct methods of attaining hexarchy, however, occur in these seedlings.

(i) In four seedlings each of the four lateral xylem strands forms a pole independently, thus producing hexarchy. This structure persists to the root tip in three seedlings, but in the other reduction to pentarchy occurs later. In one seedling of this type, one pole in the cotyledonary plane is organized very slowly, and at some levels the structure recalls that described in section iv of the pentarch types, but the xylem group is never absent in this seedling.

(ii) In two seedlings hexarchy appears only in the lower portion of the hypocotyl and the root. In transition two of the lateral xylems fuse to form one pole in the intercotyledonary plane whilst each of the lateral strands on the other side of the axis forms a pole independently, so that pentarch structure results. At a lower level in the hypocotyl one phloem group on the hitherto normal side of the axis divides and a new protoxylem appears, this becoming associated with metaxylem elements and constituting a sixth pole.

(iii) In the remaining seedling the structure demands somewhat more

detailed description. One cotyledon behaves normally, a median protoxylem group appearing in the vascular strand at the base of the petiole, but in the other cotyledon the appearance of a median protoxylem (Fig. 10) is followed quickly by the development of a second in a lateral position. In both cotyledonary bundles the two lateral xylem groups separate in the normal manner (Fig. 11). The behaviour of the strands in transition will be most easily understood by reference to the diagrams (Figs. 12 and 13). Each of the two median cotyledonary protoxylems (*a* and *e*) forms a pole; the lateral strands *f* and *g* remain independent and each forms a pole; the



FIGS. 10-13. Diagrams showing transition in a dicotylous seedling with hexarch structure due to independence of lateral strands and development of an accessory pole (*b*).

two laterals *c* and *d* on the other side of the axis fuse to form a fifth pole whilst the supernumerary protoxylem forms the remaining pole of the hexarch root.

The structure of this seedling differs from that described in section iii of the pentarch types in the two following respects:

(*a*) In the abnormal cotyledonary bundle of this seedling one of the protoxylem groups lies in the median plane and the other in a lateral position, whilst in the pentarch type both protoxylem groups arose in a lateral position, one on either side of the median plane (Figs. 4 and 11).

(*b*) In this seedling the two protoxylem groups are not organized simultaneously as in the pentarch type, the median group appearing first.

In view of the difference in position of the protoxylem groups in the two seedlings, it is suggested that the two strands in the hexarch type may be interpreted as a median and an accessory strand, instead of being regarded as the products of bifurcation of a median strand as in the pentarch type. This interpretation is supported by the fact that similar strands are found

in the polycotylous seedlings in which the median strand has also undergone bifurcation.

The slight variation in level of development of the protoxylem elements in the two strands of the hexarch seedling is not regarded as having great significance.

Another dicotylous seedling seems to approach this one somewhat in structure, since a protoxylem group is developed between the lateral xylem and the median protoxylem, whilst the overlying phloem group bifurcates. The protoxylem elements, however, quickly disappear, and the lateral xylem moves outwards to form a pole, the corresponding lateral of the other cotyledon behaving similarly.

#### THE STRUCTURE OF POLYCOTYLOUS SEEDLINGS.

The polycotylous seedlings are of the usual types, hemitricotyls, tricotyls, hemitetracotyls, and tetracotyls being found, the last two types, however, appearing only in small numbers.

##### A. *Hemitricotyls.*

In the material examined all degrees of fission are found ranging from a slight lobing of the lamina to a deep cleft extending almost to the base of the petiole. Variations in behaviour of the vascular strands have been observed, but these are in no way correlated with the degree of fission.

(i) In the majority of the hemitricotylous seedlings the strands of the two halves of the divided cotyledon unite in the petiole to form the characteristic kidney-shaped bundle in which the normal median protoxylem group appears. Careful study of the xylem elements shows that both strands contribute to the formation of the median pole so that the seedlings of this group show the type of fission which has been previously termed 'Type  $\alpha$ ' (12), i.e. the cotyledon undergoes qualitative fission.

In two examples of this type the behaviour of the lateral xylem strands is worthy of note. In one seedling each lateral strand forms a root pole independently so that the hypocotyl and the upper portion of the root possess hexarch structure, this being reduced to pentarchy ultimately by fusion of poles. In the other seedling the two laterals on one side alone form independent poles, so that pentarch symmetry obtains throughout the hypocotyl and root.

(ii) In one seedling each of the bundles supplying the two portions of the bifid cotyledon develops a median protoxylem group. A lateral xylem strand is detached on the outer side only of each bundle so that the hypocotyl and root are pentarch, three poles being formed from median strands and two from fused laterals. The lateral strands on one side of the axis are markedly unequal in size. This seedling may therefore be regarded as belonging to the class which has been termed 'Type  $\beta$ ' (12), i.e. with a quantitative fission of the cotyledon.

(iii) The *Althaea* material furnishes proof that cotyledons, in addition to the two types of equal fission previously mentioned, may also divide unequally.

In one seedling this is evident both from the external morphology and from the behaviour of the vascular strands. The abnormal cotyledon is cleft almost to the base of the lamina into two unequal portions, and it is obvious that the smaller lobe is supplied by one of the principal lateral veins, whilst the other contains the midrib and the second main lateral (Fig. 14).

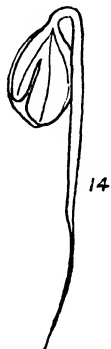


FIG. 14. Hemitricotylous seedling. The seedling is still young so that the cotyledons are unexpanded and the hypocotyl still bent. One lobe of the abnormal cotyledon (which is shown in the drawing) is supplied by a lateral strand, the other by the median strand and the second lateral.

From an anatomical study of the seedling it is clear that the strand supplying the smaller lobe behaves as an ordinary lateral strand, and transition is accomplished in a perfectly normal manner, tetrarch symmetry obtaining in the hypocotyl and the root.

In other specimens the cotyledon is more deeply cleft and the origin of the lobes is not always evident from the external morphology. A study of the vascular strands demonstrates, however, that the bundle supplying one cotyledon lobe behaves as a lateral. This point cannot be established without careful inspection of the xylem elements of numerous consecutive sections as the bundles become very closely approximated in the base of the petiole.

In one of these seedlings the lateral strand supplying the lobe forms a pole independently as does also the corresponding lateral of the normal cotyledon, but in the other seedlings the strand fuses with its fellow lateral to form one pole in the intercotyledonary plane, and it is interesting to note that this pole may be organized at a higher level than the corresponding pole on the other side of the axis.

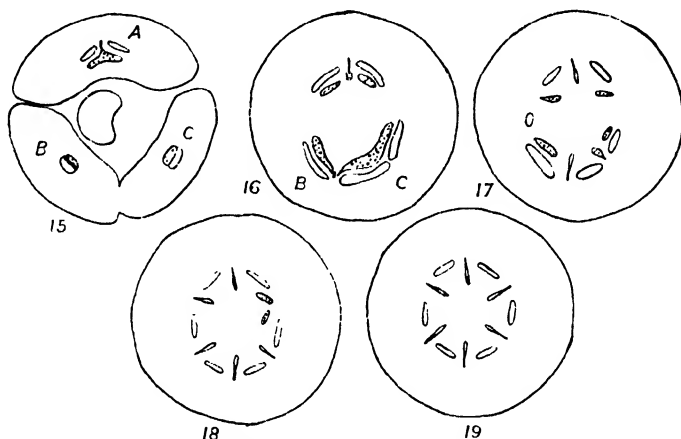
### B. *Tricotyls.*

(i) In two seedlings it is evident from the behaviour of the cotyledonary strands that the tricotylous condition has been produced by the 'Type a' mode of cotyledonary fission. In each seedling the xylem strands of two of the cotyledons give off a lateral xylem on the outer side only, whilst the inner portions of the two xylems unite to form one pole which lies in the same plane as the pole formed by the median strand of the third cotyledon. With this type of cotyledon structure tetrarch symmetry is to be expected, but this is not realized in either case because of peculiarities in behaviour of the lateral xylem strands. In one seedling

the two lateral strands on one side of the axis unite to form a pole in the intercotyledonary plane, but on the other side each strand forms a pole, so that pentarchy prevails in both hypocotyl and root. In the other seedling there is a short pentarch phase, but the two poles formed by independent laterals soon undergo fusion. At a lower level in the hypocotyl, however, a new pole arises in the diagonal plane and the pentarch structure thus established persists throughout the root.

These tricotylous seedlings therefore show peculiarities in transition similar to those described for some dicotylous seedlings.

The structure of a third tricotylous seedling will be described in



FIGS. 15-19. Diagrams of transition stages in a tricotylous seedling. The strands of two cotyledons unite to form one root pole. Hexarchy results from independence of lateral strands, and development of an accessory pole.

greater detail. The strand from one cotyledon behaves normally, the phloem bifurcating whilst the xylem divides into a median and two lateral portions, but the other two cotyledonary strands retain their collateral structure (Fig. 15). Just below the cotyledonary node these strands (B and C, Fig. 16) approach one another and a xylem pole is organized between them, both groups contributing to its formation. Almost immediately the phloem of strand C divides and a second xylem pole is formed (Fig. 17). The outer portion of the xylem of both B and C becomes separated, forming lateral groups in the normal way. The lateral xylem of bundle C fuses with the lateral strand from bundle A, but the other lateral of A and the lateral of B remain apart and each forms a pole. The hypocotyl is therefore hexarch in structure (Figs. 18 and 19).

It is suggested that this seedling may be regarded as one in which the two cotyledons B and C have been produced by the 'Type a' mode of fission, the xylem pole produced by the strands from both cotyledons corresponding to the median pole formed by the strand of an undivided

cotyledon. The transition is complicated, however, by the presence of an accessory pole in one half of the cotyledon, and by the fact that two of the laterals form poles independently. According to this interpretation the structure is identical with that of the dicotyl described in section iii of the hexarch types (compare Figs. 11–12 with Figs. 17–18).

(ii) Four tricotylous seedlings exhibit the 'Type  $\beta$ ' mode of fission. One specimen is almost perfectly symmetrical in the upper region of the hypocotyl. The strand from each cotyledon gives rise in the hypocotyl to a median pole and two lateral strands. Two pairs of lateral strands fuse to form two poles, but the members of the third pair are unequal in size and the pole is formed by the larger one alone, the smaller strand disappearing just prior to fusion. At a lower level in the seedling pentarchy obtains owing to the disappearance of one pole.

In a second seedling two of the cotyledonary strands give rise to a lateral on one side only so that pentarch structure is produced from the outset.

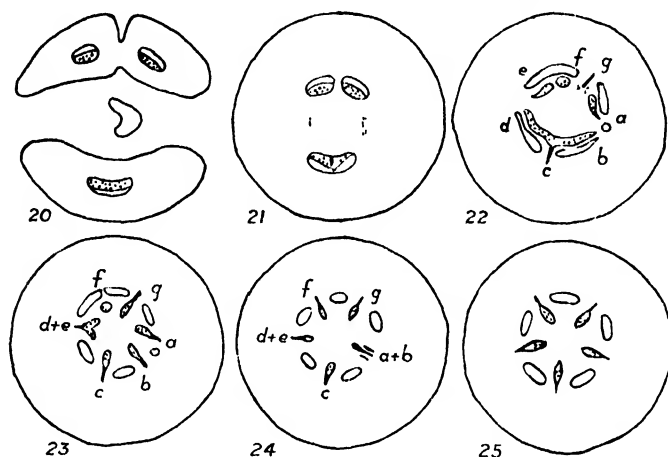
The third seedling has suffered considerable injury, and one cotyledon strand is very small, this producing no lateral groups at all. The lateral strands produced from the other two bundles on the side nearest the damaged cotyledon form independent poles so that hexarchy obtains, this being followed later by pentarchy through the disappearance of one pole.

In the fourth seedling two cotyledonary strands give off no lateral xylem groups on their adjacent sides, and only very small ones on the outer sides. Two intercotyledonary poles only are formed, these consisting principally or entirely of the lateral strands derived from the third cotyledon. The seedling is therefore pentarch.

(iii) In one seedling the structure seems to indicate that the tricotylous condition may have been produced by the unequal fission of one cotyledon. Two of the cotyledon petioles fuse slightly early, and the strands supplying these cotyledons become closely approximated in the hypocotyl. From one strand two poles are organized ( $g$  and  $a$ , Fig. 22), each appearing near the outer edge of the xylem mass. The other strand divides into two parts ( $e$  and  $f$ , Fig. 22) one of which ( $f$ ) consists of a few metaxylem elements only. The vascular bundle belonging to the third cotyledon behaves normally, forming a central protoxylem group and two lateral groups ( $d$ ,  $c$ ,  $b$ , Fig. 22). The one lateral strand  $d$  of the normal cotyledon unites with strand  $e$  to form a pole, whilst the other lateral  $b$  forms a pole independently. This pole, however, later undergoes fusion with pole  $a$ , this being accomplished in the special way described for some dicotyls (cf. p. 371). A median xylem group appears between poles  $a$  and  $b$ , and the metaxylem elements of these poles unite with the median group whilst the protoxylem elements occupy an isolated position on either side (Fig. 24). Ultimately the protoxylem elements disappear or fuse with the central protoxylem. The

phloem group originally lying between poles *a* and *b* has meanwhile disappeared. During the fusion of *a* and *b* the group of metaxylem elements *f* (Fig. 23) acquires a protoxylem whilst the overlying phloem group divides. The lower portion of the hypocotyl therefore and the root are pentarch (Fig. 25).

The two abnormal cotyledons are regarded as produced by unequal fission of one cotyledon, pole *g* corresponding to the median pole of this cotyledon. Strands *e* and *a* are interpreted as the lateral strands *e*, behaving normally and fusing with the corresponding lateral from the other



FIGS. 20-5. Diagrams of transition stages in a tricotylous seedling in which tricotily has arisen by the unequal fission of a cotyledon. An accessory pole is developed.

cotyledon, whilst *a* at first forms an independent pole and only later undergoes fusion with pole *b*, formed by the corresponding lateral of the normal cotyledon. Pole *f* is interpreted as an accessory pole (compare dicotyl, Figs. 10-13).

### *C. Hemitetracotyls.*

(i) In two seedlings each cotyledon is cleft nearly to the base and has apparently divided according to the 'Type a' method. The strands from the two lobes of each cotyledon fuse at the base and behave like the strand of a normal cotyledon, a central protoxylem and two lateral groups of xylem being organized. The laterals fuse in pairs and tetrarch symmetry obtains in the hypocotyl and root.

Two other seedlings agree with those just described in that each cotyledon has undergone type *a* fission, but one strand in addition to contributing to the median cotyledonary pole, and giving off a lateral xylem group on the other side, forms an extra pole so that the hypocotyl and root are pentarch.

(ii) In two seedlings each cotyledon has divided in the 'Type  $\beta$ ' manner so that the strand supplying each lobe forms a median protoxylem group. A lateral group of xylem is given off on one side only of each strand. The laterals fuse in pairs forming two root poles, so that the hypocotyl is hexarch. Reduction to pentarchy takes place in one seedling in the hypocotyl by the disappearance of one intercotyledonary pole, and in the other seedling in the root by fusion of poles.

A third seedling is of the same general type but differs in one or two details. As in the other two specimens each bundle forms a pole, but in two of the strands the bifurcation of the phloem and the assumption by the protoxylem of an exarch position occurs at a somewhat lower level than usual. A lateral strand is given off on the outer side of each bundle, the four strands so produced fusing in pairs to form two poles in the intercotyledonary plane. The hypocotyl is therefore hexarch. One of the bundles which 'rotates' at a low level gives off also a small xylem strand on the side nearest its fellow bundle. This strand, which never consists of more than three or four elements, persists for a short time between the two bundles but disappears before the organization of pole structure is complete.

This seedling also shows ultimate reduction to pentarchy by fusion of poles.

(iii) In three seedlings unequal fission of one cotyledon has taken place, and since other structural peculiarities are found each must be briefly described.

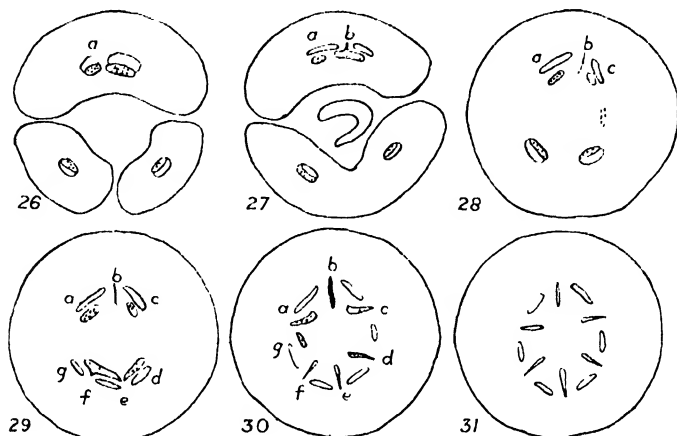
*Seedling A.* One of the bifid cotyledons which is cleft nearly to the base of the lamina has undergone 'Type  $\alpha$ ' fission; the two strands unite in the petiole to form a normal kidney-shaped bundle in which a median protoxylem group and two lateral xylem groups are organized. In the other cotyledon the cleft extends to the upper part of the petiole. The xylem strand supplying one portion gives rise to an asymmetrically developed pole and to a lateral xylem group which ultimately fuses with the corresponding lateral of the other cotyledon. The strand supplying the second lobe of the cotyledon forms one pole and is interpreted as a lateral strand. The corresponding lateral of the other cotyledon also forms a pole independently so that the hypocotyl is pentarch.

*Seedling B.* This has one cotyledon divided to the base of the lamina whilst the fission extends to the petiole in the other. In the first cotyledon the one strand behaves as a lateral (Fig. 26, *a*), whilst the other gives rise to an asymmetric pole *b*, and a lateral xylem group *c* (Fig. 28). In the other cotyledon the two bundles approach one another and the adjacent xylem elements form a central pole (Fig. 29, *e*), whilst a lateral strand is given off on the outer side in each case (*d* and *g*, Fig. 29). In addition, one bundle gives rise to an extra pole *f*, lying between the lateral strand and the

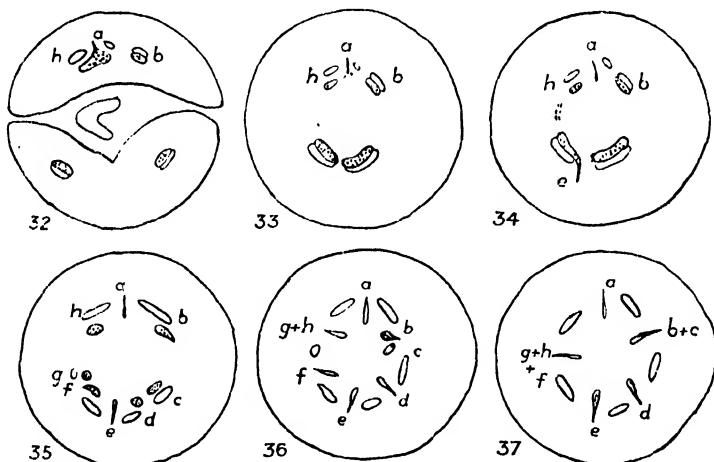


median pole. Lateral strands *a* and *g* fuse although *g* becomes very small before fusion takes place, but laterals *c* and *d* remain independent, each forming a pole. The hypocotyl is therefore hexarch (Fig. 31).

*Seedling C.* In one cotyledon the two strands supplying the lobes



FIGS. 26-31. Diagrams illustrating transition in Hemitetracotyl B with unequal fission of one cotyledon; independence of laterals (*c* and *d*); development of an accessory pole (*f*).



FIGS. 32-37. Diagrams illustrating transition in Hemitetracotyl C, showing unequal fission of both cotyledons, and development of an accessory pole (*a*).

become closely associated in the petiole, but it is clear that one gives rise to an asymmetric pole *a* (Fig. 32) and a lateral strand *h*, whilst the other forms a lateral strand only (Fig. 32, *b*).

Of the strands supplying the other lobed cotyledon one gives rise to an asymmetric pole *e* and another group of xylem which moves laterally and acquires an exarch protoxylem (*f*). The other bundle divides into

two portions one of which behaves as a lateral strand (*c*), whilst the other (*d*), after some time, acquires protoxylem elements. The two lateral strands *b* and *c* fuse in the normal way. Strand *f* at first forms an independent pole and also gives off two or three elements (group *g*, Fig. 35) to bundle *h* which also develops an exarch protoxylem. Finally, however, the intervening phloem group disappears and strand *f* fuses with the strand *g* + *h* (Figs. 36, 37). The structure of this seedling is somewhat difficult to interpret, but it seems most probable that *e* is a median pole and that strand *f* is a lateral strand which at first tends to remain independent, but later fuses with the corresponding lateral *h* from the other cotyledon. Pole *d* is interpreted as an accessory pole.

#### D. *Tetracotyls*.

Five seedlings only of this type have been examined.

(i) In one seedling the strands belonging to two cotyledons become associated below the cotyledonary node, the adjacent elements forming a median pole, whilst two lateral xylem strands are produced. These were therefore produced by 'Type  $\alpha$ ' fission.

The strands of the other two cotyledons also become associated and form a median pole and two lateral xylems, but on one side an additional pole is produced between the lateral xylem and the median pole. The two lateral xylems on this side of the axis unite to form one pole, but the other two laterals form root poles independently so that in the hypocotyl hexarch structure obtains. Reduction to pentarchy takes place ultimately by the disappearance of one of the poles formed by a single lateral strand.

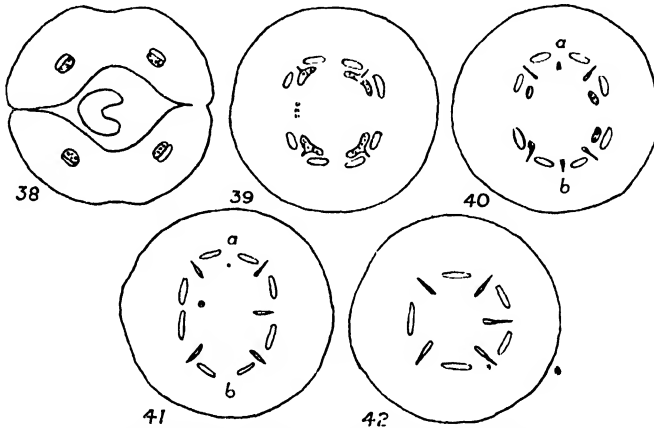
(ii) In a second seedling the bundles of two cotyledons produce a median pole and two lateral xylem strands. The bundle of each of the other two cotyledons forms a pole and one lateral strand only, this being produced on the side away from the neighbouring bundle. Of the four lateral strands present in the hypocotyl two unite to form a pole whilst the others remain independent, so that the hypocotyl has hexarch structure. Reduction to pentarchy occurs in the extreme root tip. In this seedling, therefore, two cotyledons have been produced by 'Type  $\alpha$ ' fission and two by 'Type  $\beta$ ' fission.

The third seedling is exactly like the preceding one except that the lateral of the type  $\beta$  cotyledon, which remained independent in the seedling just described, is not present. The upper portion of the hypocotyl therefore contains five xylem poles, but a pole soon appears in the position which should have been occupied by the lateral strand and hexarchy obtains from this point downwards.

(iii) In the fourth seedling the bundle of each cotyledon develops a median protoxylem and the phloem bifurcates (Fig. 39). The four protoxylem groups form the corners of a rectangle. In the middle of each

of the shorter sides of the rectangle a xylem pole is quickly organized, the constituent elements being derived from both the adjacent bundles (Fig. 40, *a*, *b*). Two lateral xylem groups become detached on one of the longer sides of the rectangle and unite to form a pole, but on the other side only one lateral strand is developed and this, although it ultimately moves into a position midway between the two adjacent poles (Figs. 40, 41), dies out without any attempt at true pole formation.

The two poles *a* and *b* (Figs. 40, 41) are never large and are quickly



FIGS. 38-42. Diagrams illustrating transition in a tetracotylous seedling. The median strand of each cotyledon forms a root pole, but the lateral strands are of minor importance.

reduced to two or three protoxylem elements. These are fairly persistent so that for a time the hypocotyl may be described as heptarch, but ultimately the protoxylem elements die out and pentarchy obtains throughout the lower portion of the seedling (Fig. 42).

The fifth tetracotylous seedling resembles very closely the one just described, but in this case both intercotyledonary poles are organized on the longer sides of the rectangle formed by the vascular tissue. The seedling is never really octarch, however, because one of the other two intercotyledonary poles (that is, those corresponding to poles *a* and *b* of Fig. 40) disappears very early. The seedling therefore is heptarch, and later, hexarch.

#### DISCUSSION.

The discussion may be divided conveniently into two sections, A and B: the one dealing with the variations in vascular symmetry which are found in both dicotylous and polycotylous seedlings, and which are apparently independent of any variation in cotyledon number; the second with the phenomenon of polycotily and the associated changes in the vascular system of the seedling.

A. The most striking feature of the dicotylous seedlings examined is the tendency shown towards pentarch symmetry and the variety of ways in which this may be attained. Hexarch structure is apparently of comparatively rare occurrence.

The production of either a tetrarch or a hexarch root in the seedlings of one species was recorded by Dr. E. N. Thomas (17) for *Pyrus communis*. The hexarch condition in this species is due to independent pole formation by the lateral cotyledonary strands, although these may be fused with the median strand in the base of the cotyledon petiole just as in *Althaea rosea*. Gérard (8) in a very brief description of the seedling stated that *Pyrus communis* had a pentarch root, one root pole being associated with an epicotyledonary strand. Such participation by epicotyledonary strands in root-pole formation is certainly not typical of the species, and the observation seems to require confirmation.

Compton (5) described seedlings of *Vicia Faba* in which the root was either tetrarch, pentarch, or hexarch, the pentarch condition being apparently the most usual. The fifth pole, however, was present in the lower portion of the seedling only, the protoxylem dying out at higher levels whilst the metaxylem became fused with a neighbouring cotyledonary strand.

Pentarchy has also been recorded as a rare occurrence in seedlings which are normally tetrarch. Kattein (15), for instance, described a pentarch seedling of *Helianthus annuus* which, according to his description, seems to be comparable with many of the pentarch specimens of *Althaea*. In *Helianthus* the lateral strands unite in the hypocotyl to form two endarch collateral bundles each of which later gives rise to a root pole in the intercotyledonary plane. In the abnormal specimen the protoxylem of one bundle bifurcates and each half turns outward so that two poles are formed on that side of the axis. Compton (6) assumed that the extra pole in this seedling was due to a 'Zwischenstrang' (i.e. an accessory strand occurring in the cotyledon and hypocotyl) which had fused with the lateral strands, but this is not supported by the structure of the seedling. Accessory strands have indeed been observed in *Helianthus*, and Compton (6) recorded the formation of a root pole by such a strand, but in the seedling in question the accessory strand, if present, would be fused with the laterals throughout its course until the time of root-pole organization. It seems reasonable to interpret the pentarch structure of this seedling as due to the formation of independent poles by two of the lateral strands.

In *Acer Pseudoplatanus* (14) several seedlings have been described in which the lateral strands are separated in the hypocotyl by phloem groups which may or may not be connected with the plumular strand, but the seedlings were in all cases too young to determine whether there was any effect on the number of root poles formed.

In *Althaea rosea* the pentarch condition arises in many seedlings in the same manner as pentarchy and hexarchy in *Helianthus annuus* and *Pyrus communis*, since the lateral strands form independent root poles. The cause of this unilateral modification is not obvious. It is not apparently determined by the position of the first epicotyledonary leaf since it does not invariably occur on the same side of the axis as the first leaf.

It seems possible to interpret several of the phenomena described as variants of one type of modification of the seedling anatomy. Thus, in a number of seedlings pentarchy (produced by independence of lateral strands) prevails throughout the axis; in some specimens the cotyledonary laterals tend to remain distinct, and some of their outer xylem elements linger on either side of the single intercotyledonary root pole which is ultimately formed; in one seedling, again, the lateral cotyledonary strands fuse to form one pole which undergoes bifurcation at a lower level in the axis. These phenomena can be co-ordinated if it is assumed that the unknown factor tending to produce pentarchy may affect the root alone, or the cotyledons alone, or both organs equally. If both are equally affected pentarchy will result; if the root only is affected then the structure found in the one example mentioned above will be attained; whilst if the cotyledonary strands alone are modified the phenomenon described earlier as 'transient pentarchy' will appear.

Other methods of attaining pentarchy seem to be quite distinct from those discussed above. In some seedlings pentarchy has been found to arise in the hypocotyl as the result of the development of a new xylem pole in the diagonal plane (pp. 372 and 373). In other seedlings an accessory pole appears at the base of the cotyledonary petiole and is continued downward through the hypocotyl in the diagonal plane (pp. 374 and 378). It is noteworthy that in the second group the venation of the cotyledon is not abnormal, and that the accessory pole is, with one exception to be discussed later, prolonged into the root.

It seemed, therefore, possible that the two types might be variants of one mode of increase in root-pole number. The extra xylem pole would on this view be regarded as originating in the root and as being prolonged upward to a varying extent in different individuals. In some cases it may cease to be developed in the hypocotyl, whilst in others it may be prolonged to the base of the cotyledon. In this connexion it is of interest to note that whilst such accessory poles are fairly numerous among the polycotylous seedlings, in all cases the pole extends nearly to the cotyledon petiole or may be prolonged into it. On the other hand, in the dicotylous specimens the pole more frequently fails to develop above a fairly low level in the hypocotyl. Whether this difference of behaviour can be correlated with the greater importance which such strands might be assumed to possess in polycotylous seedlings as accessory conducting strands is doubtful in view

of the general absence of correlation between the importance of strands functionally and their behaviour in transition.

Some difficulty is found in applying the interpretation outlined above to one exceptional seedling which has been examined in which the accessory protoxylem is present at the cotyledonary node and immediately below, but then disappears. The seedling is exceptional also in the fact that this accessory protoxylem appears on the same side of the axis as two independent lateral strands, whereas in all other seedlings examined, if accessory poles and independent laterals occur in the same seedling they are on the opposite sides of the axis.

It might be suggested that the development of accessory poles could originate either in the cotyledon or in the root. Compton (6) has recorded in *Helianthus* instances of accessory strands being present in the cotyledon lamina and hypocotyl, and exceptionally prolonged into the root as an extra root pole. In the seedling in question, however, there is no abnormality in the venation of the cotyledon. A possible alternative explanation is that the unusual behaviour of the accessory strand is connected with the presence of two independent lateral strands on the same side of the axis, but it does not seem probable that the independence of laterals would affect the accessory pole in this particular manner.

In one dicotylous seedling an interesting approach to diagonal tetrarchy is evident, since each of the four lateral strands forms a root pole, whilst one median cotyledonary strand fails to organize a pole in the upper portion of the hypocotyl. The root pole in the cotyledonary plane appears, however, at a lower level, and hexarch structure obtains throughout the remainder of the seedling.

There remains to be discussed the dicotylous seedling in which the median strand of one cotyledon is associated with two protoxylem groups. In the literature dealing with polycotylous seedlings instances have been recorded in which the bifurcation of the cotyledonary strand was accompanied by fission of the cotyledon lamina (Compton (6), Hill and de Fraine<sup>1</sup>(10), Bexon (1)), but in such cases the cotyledon midrib was double, and these forms have been interpreted (1) as resulting from a secondary fusion of the laminae. In the seedling under discussion, however, the midrib of the cotyledon is not double in the lamina, the two protoxylem groups merely appearing in the petiole at the beginning of transition. Apparently in this seedling there is the same tendency towards increase in root-pole number by bifurcation, as is shown by other *Althaea* seedlings (e.g. pentarchy, section v, p. 373), but the pole affected in this seedling is one in the cotyledonary plane, instead of in the intercotyledonary plane. Such increase in root-pole number may be, as has been shown, unassociated with any change in the vascular system of the upper portion of the seedling.

The cause of the great variability of structure in *Althaea rosea* is unknown. It does not seem to be based on any obvious physiological necessities for the behaviour of a strand in transition does not show any close correlation with its importance as a conducting strand. Thus, a lateral strand of a normal cotyledon may form an independent root pole, whilst, when supplying a lobe of a lobed cotyledon, it may fuse with the corresponding lateral strand of the other cotyledon to form one pole. Again, hexarchy does not seem to be characteristic of the Malvaceae so far as the seedling structure of this family has been investigated. Chauveaud (4) has described *Malva sylvestris* as having typical tetrarch structure. In connexion with the present investigation, seedlings of species of *Gossypium*, *Malva*, *Malope*, *Lavatera*, and *Hibiscus* have been examined, and although the examination has not been so detailed as in *Althaea*, it seems evident that these forms have normally tetrarch symmetry. If, therefore, the tendency towards pentarch and hexarch structure is to be interpreted as due to descent from hexarch ancestors, *Althaea* must be assumed to have retained a character which has been lost by its allies. The great variability displayed in method of attaining pentarch and hexarch structure may perhaps be regarded as militating against the theory of survival of an ancestral character.

The structure displayed by the dicotylous seedlings seems to lead to the conclusion that modification of the seedling vascular system may originate in either the lower or the upper portion of the seedling, and may affect one part only. This does not, however, seem incompatible with the view that the seedling vascular system may be regarded as a single whole, on which factors leading to modification may act in varying degrees. The alternative view advocated by Bugnon (3) that the vascular system of cotyledon and root must be regarded as two distinct systems has been recently shown by Thomas (18) to fail in interpretation of all the facts observed, notably in interpretation of the structure of the epicotyledonary strands.

B. Very little work has been done previously on polycotily in seedlings possessing normally tetrarch symmetry. Gain (7) examined polycotylous specimens of *Phaseolus*, which were found to possess pentarch or hexarch structure, but the course of the individual strands was not accurately followed. Léger (16) studied *Acer platanoides* and found pentarchy and hexarchy in the tricotylous specimens, and heptarchy in tetracotyls. He found that each of the cotyledons produced by fission may be associated with a separate root pole. The behaviour of the lateral strands is of interest. Léger found that in tricotylous seedlings the adjacent lateral strands of the two cotyledons arising by fission might not form a root pole, in which case the root was pentarch, or they might be sufficiently important to form a pole so producing a hexarch root.

A single tricotylous specimen of *Impatiens Roylei* has been described (13) but this seems to be distinctly aberrant in structure.

The majority of the polycotylous seedlings of *Althaea rosea* investigated, apart from the peculiarities which they have in common with the dicotylous seedlings, are of a type similar to those found in other species. On the one hand, the median strands of two cotyledons or cotyledon lobes may unite and behave in transition like the median strand of a single cotyledon, or on the other hand, each cotyledon may be associated with a separate root pole. In seedlings of the second type the adjacent lateral strands of the cotyledons resulting from fission may or may not form an intercotyledonary root pole. *Althaea*, therefore, in this respect, shows the same types of behaviour as were found by Léger in *Acer platanoides*.

The chief point of interest in the polycotylous material is found in the proof of the existence of unequal fission in some cotyledons. This had been previously suggested as a possible method of cotylar increase (9), but the evidence hitherto available was insufficient to establish definitely the fact of its occurrence. The behaviour of the lateral strands in such seedlings is of interest. It is very striking to note that whilst in dicotylous seedlings the lateral strands may remain separate and form independent root poles, in the polycotylous specimens a lateral strand which is supplying a separate lobe or (if the cotylar cleft is very deep) almost a separate cotyledon, may fuse with the corresponding lateral from the normal cotyledon to form one root pole. It seems evident that an increase in importance of a strand in the cotyledon lamina such as presumably results from such fission may have no effect on its behaviour in transition.

These seedlings may also furnish some evidence concerning cotyledons described by the writer (1, 2) and by Hill and de Fraine (9, 11) which are peculiar in that their median vascular strand does not take part in root-pole formation. One possible interpretation of such cotyledons is that they may be produced by asymmetrical fission of the parent cotyledon, and that the median strand behaves abnormally because it is the equivalent of a lateral strand.

In *Althaea*, where there are cotyledons or cotyledon lobes, which are undoubtedly supplied by lateral strands, these behave like the lateral strands of normal cotyledons, fusing with the median strand and either forming a pole after union with another lateral, or forming a pole independently. The behaviour of these undoubted lateral strands does not give any support to the idea that the strands in the peculiar cotyledons in question are of the same type, since the strands may be prolonged for some distance in the hypocotyl of seedlings in which laterals are normally absent or of little significance.

In conclusion it may be said that the study of *Althaea rosea*, whilst



it raises several problems for which there is no obvious solution, is of interest in that it shows

(a) the wide range of types of structure which seedlings of one species may exhibit, e.g. cruciform tetrarchy, pentarchy, hexarchy, and some approach to diagonal tetrarchy.

(b) the slight degree of correlation between the functional importance of strands and their behaviour in transition.

It seems very probable that *Althaea* is not an isolated example of such variability, but that seedlings of other species if examined in sufficient detail might show similar variability.

#### SUMMARY.

1. The usual tetrarch structure of the dicotylous seedling is described.
2. Pentarch dicotyls are described, the pentarchy resulting from
  - (a) independence of lateral strands.
  - (b) development of an accessory pole in the diagonal plane.
  - (c) the bifurcation of a root pole in the intercotyledonary plane.
  - (d) the bifurcation of a root pole in the cotyledonary plane.
  - (e) the independence of laterals associated with the failure of one pole to develop in the cotyledonary plane.
3. Dicotylous seedlings are found to show hexarch structure, this being produced by the independence of laterals alone or combined with the development of an accessory pole.
4. Polycotylous seedlings are described which show vascular abnormalities similar to those described above, together with the vascular modifications usually accompanying the polycotylous condition.
5. In addition to the types of polycotily found in other species, some specimens are polycotylous through an asymmetrical fission of the cotyledon lamina.

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# On the Nature of the Resistance of the Potato to Wart Disease.

BY

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With Plate XIV.

THIS investigation was undertaken in the hope of throwing some light on the nature of immunity to wart disease exhibited by certain varieties of potato. The main object was to determine whether infection of immune varieties took place at all, and, if so, in what way the development of disease was stayed. An examination also was made of shoots of both immune and susceptible varieties in order to ascertain whether there were any differences, such as thickness of cuticle, wax formation, growth of hairs or any other feature which might form an obstacle to penetration by the parasite.

## *Examination of Uninfected Shoots.*

The following varieties were examined :

<i>Susceptible.</i>	<i>Immune.</i>
Arran Chief.	Great Scot.
Midlothian Early.	Tinwald Perfection.
Ninety Fold.	Edzell Blue.
	Kerr's Pink.

Only very young shoots, less than  $\frac{1}{4}$  in. high, were used, hand sections being made from fresh material and microtome sections from material fixed in Flemming's solution. A variety of cuticle stains were tried: scharlach red in 70 per cent. alcohol, sudan III, ammoniacal fuchsin, and chlorophyll, freshly extracted in alcohol. Of these four, scharlach red was found to give the most satisfactory results, staining the cuticle a faint red; this showed more clearly when lichtgrün was used as a counter stain. No anatomical differences were observed between the two sets of shoots and no difference in staining reactions could be detected between the cuticle of susceptible and immune varieties; in fact, very little cuticle appeared to

shoots of Arran Chief. The rest of the shoots of Arran Chief were left to act as controls, the development of warts being taken as an indication of the success of the inoculation. It was considered useless to examine the fixed material unless the controls gave a high percentage of infection. The fixative used was Flemming's strong solution diluted with water to half its strength. For staining gentian violet and orange G. were usually employed and occasionally Heidenhain's iron-alum-haematoxylin.

In the sections of the shoots of Great Scot not only were zoospores found fixed to the surface of the epidermis (Pl. XIV, Fig. 1), but others were to be observed passing through the cuticle and outer wall of the epidermal cells (Pl. XIV, Figs. 2-7). In one case a zoospore was seen half-way through the wall, the part inside being connected with the part outside by a fine thread very darkly staining and showing all the more clearly because of the thickness of the wall; Pl. XIV, Fig. 5 shows the strand through the wall running to the nucleus of the zoospore. Pl. XIV, Fig. 8 shows the zoospore immediately after entry.

Comparison of these sections with sections through similar infected material of Arran Chief (Pl. XIV, Figs. 13-16) showed beyond doubt that these were zoospores of *Synchytrium endobioticum* penetrating the tissues of the variety Great Scot; they agreed in size and method of penetration with those figured by Miss Curtis. Material of Great Scot fixed three days after inoculation showed inclusions in the cells with every appearance of being the parasite reduced in size and apparently becoming disorganized (Pl. XIV, Figs. 9-11). These inclusions had a definite structure consisting of a reticulated body with a distinct nucleus surrounded by a clear space. The reticulated part was shrunken and appeared to be in process of dissolution. The nucleus was small in size and sometimes faintly staining. In no case was the parasite found in any later stage of development, and no warts were seen. The parasite apparently dies after entry without bringing about the death of the host-cell.

It is evident that immunity to wart disease, at least in the variety Great Scot, does not depend on a capacity to keep the invader out, but is mainly or wholly due to some physiological characteristic of the cells which render them unsuitable for the further development of the parasite. In the present state of our knowledge it is hardly profitable to speculate on the nature of this characteristic.

#### SUMMARY.

There is no anatomical difference between the young shoots of varieties of potato respectively immune and susceptible to wart disease.

Changes of temperature had very little effect on the degree of infection. Infections were obtained from temperatures varying from 58° to 80° F.

The healthiest tubers took the disease most readily. The most satisfactory infections were made with tubers kept under normal conditions.

Zoospores of the parasite are capable of penetrating the epidermal cells of young shoots of the immune variety Great Scot. For the first two days the development of the organism in immune varieties seems normal: the organism increases in size and travels down the cell in the same manner as it does in susceptible varieties. After that time it becomes smaller and less definite in outline, showing signs of disorganization. It appears finally to become dissolved and so disappears from the host-cell.

The resistance of the immune variety Great Scot is not due to a capacity to prevent the entry of the invading parasite, but to some physiological characteristic of the epidermal cells which brings about the death of the invader after entry.

This work was undertaken at the suggestion of Professor V. H. Blackman, whom I wish to thank for kindly criticism.

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#### DESCRIPTION OF PLATE XIV.

Illustrating Mrs. K. Cartwright's paper on the Nature of the Resistance of the Potato to Wart Disease.

Figs. 1-8. Stages of infection of the epidermis of the immune variety Great Scot.

Fig. 1. Zoospore on surface with cilium partly retracted. The host-cell cytoplasm shows a dark staining mass just opposite the zoospore.  $\times 1,625$ .

Fig. 2. Early stage of penetration; section slightly oblique.  $\times 1,625$ .

Fig. 3. The upper zoospore shows a peg-like structure connected with the nucleus, penetrating the cell-wall. The nuclear contents of the lower one are within the cell.  $\times 1,500$ .

Fig. 4. An indication of a thread-like structure connected with the nucleus of zoospore is to be seen through the cell-wall.  $\times 1,750$ .

Fig. 5. A dark staining thread is clearly seen penetrating the cell-wall.  $\times 2,000$ .

Fig. 6. Host-cell cytoplasm contracted; the thread-like structure is very clearly shown.  $\times 1,400$ .

Fig. 7. Part of the contents of the zoospore is to be seen inside the host-cell connected to the nucleus of zoospore by a fine thread.  $\times 1,833$ .

Fig. 8. Penetration complete; the zoospore is inside the host-cell.  $\times 1,700$ .

Figs. 9-12. Later stages, showing the parasite inside the tissues of the variety Great Scot.

Fig. 9. The parasite shows an irregular outline and reticulated structure; has not yet passed the host-nucleus.  $\times 1,700$ .

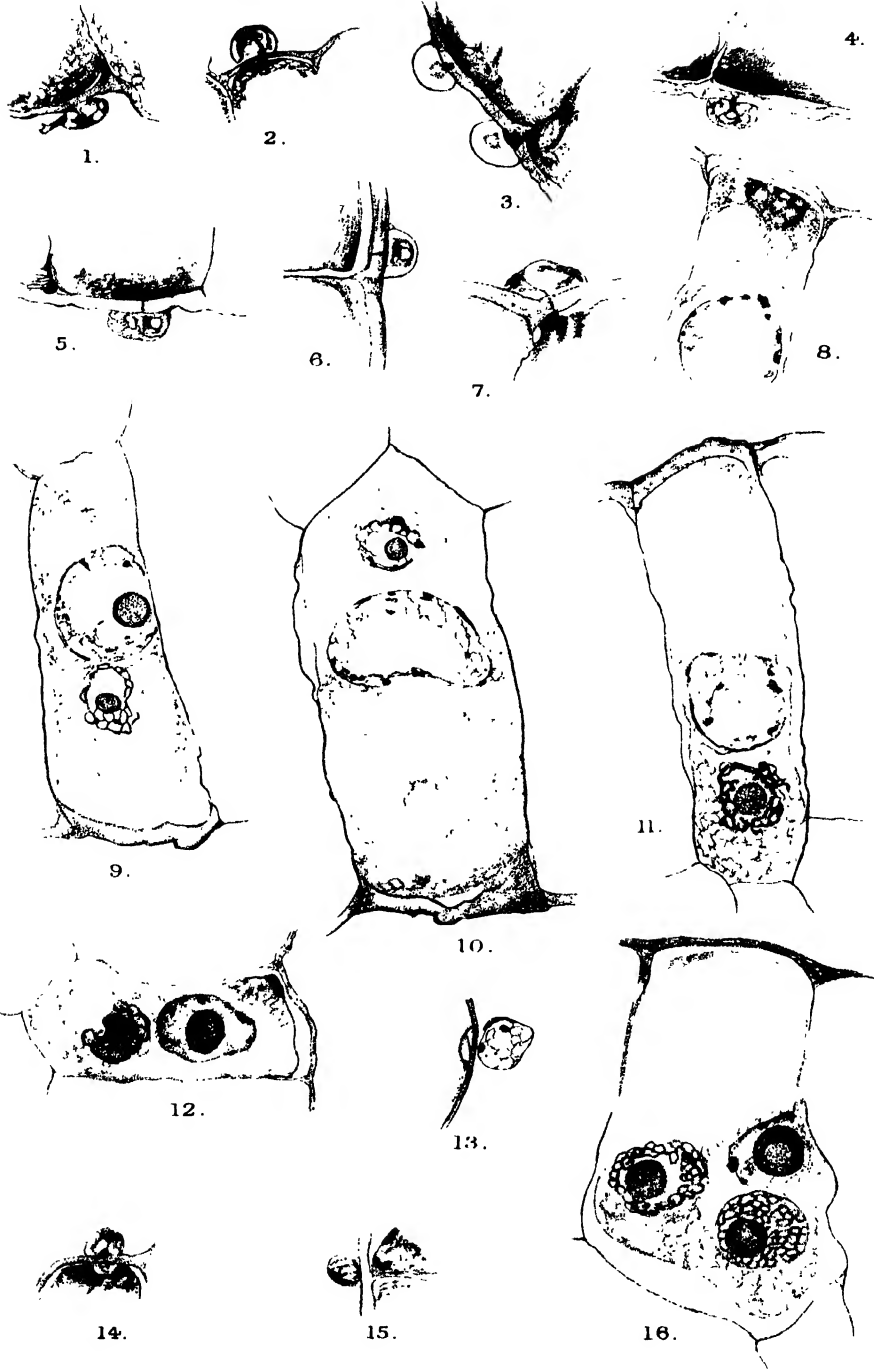
Figs. 10-12. Later stages with the parasite at the base of the epidermal cell and showing an irregular outline.  $\times 1,700$ .

Figs. 13-16. Stages of penetration into the susceptible variety Arran Chief.

Figs. 13-15. Stages of penetration.  $\times 1,850$ .

Fig. 16. Two of the parasites in one cell of Arran Chief. The more regular outline of the organisms as compared with those of Figs. 9-12 is to be noted.  $\times 1,475$ .





Huth, London.





# The Mechanical Action of Corticolous Lichens.

BY

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With thirty-five Figures in the Text.

OWING to the nature of their substratum, crustaceous, epiphloeodal lichens are more readily sectioned *in situ* than epilithic forms, and by making a preliminary study of the former it was thought to gain an insight into the more difficult subject of the mechanical action of the latter on substrata.

The following is not by any means a complete anatomical study of the bark lichen types, but is rather an investigation of the effect produced by such plants on a substratum of bark.

## I. PREPARATION OF MATERIAL.

Lichens growing on thin, unbroken, smooth bark of young twigs of such trees as *Quercus*, *Fraxinus*, *Crataegus*, &c., were generally selected for this investigation. Owing to the nature of the cork, the material offered difficulties in infiltration with paraffin wax, and in mounting. The celloidin method was not attempted since serial sections were required. The method finally adopted was to cut away, before embedding, as much as possible of the wood and secondary cortex below the lichen, without stretching or breaking the bark, and so causing injury at the actual junction of the lichen and its substratum. In addition, in some cases, it was even found advisable to cut away still more of the periderm after the lichens were embedded in the paraffin. In this way injury to the outer bark with its adhering lichen was minimized. The material was then put back into the wax bath for a short time and re-embedded.

The material was fixed with acetic alcohol (absolute alcohol three parts, glacial acetic acid one part), usually under reduced pressure for a few hours, and then allowed to remain in the reagent over night or even for twenty-four hours. Treatment after this was of the usual kind.

Microtome sections of varying thicknesses were cut, but those of  $7\ \mu$  to  $15\ \mu$  were found most useful.

Diluted white of egg was used as a fixative, but owing to the waxy nature of the cork walls, and the readiness with which the lichen tissues absorb water, great difficulty was experienced in making the sections adhere to the slide when the paraffin wax was dissolved away. Although as little as possible of the tissues of the tree had been left below the epiphyte, yet the periderm layers remaining refused to adhere to the slide and so floated free in the xylol and alcohol, and commonly caused the whole section to become free. It was found that by scraping away with a small scalpel the greater part of the remaining cork, while the dry sections were still in the paraffin and adhering to the slide, this difficulty was considerably lessened.

Secondly, the gelatinous nature of the lichen was a source of difficulty. The tissues of the lichen swelled greatly on being brought into contact with water, so that, whereas in the xylol and absolute alcohol the lichen part of the sections remained flat, adhering to the slide, on reaching the aqueous alcohols they became loosened from the glass in many places—appearing puckered or frilled. This, together with the weakness of the suberin attachment, caused many of the sections to be lost in the earlier part of the investigation.

Heidenhain's iron-alum-haematoxylin, with congo red as a counter stain (3), was generally used, and since cytological details were not required it was found better slightly to overstain the material.

## II. PERIDERM STRUCTURE.

Before dealing with the effect of corticolous lichens on their substrata it may be as well to recall, very briefly, the microscopic structure of cork.

Tabular cork, as stated by Haberlandt (6), may have thin or thick walls, but in either case these walls are usually of uniform thickness all over, and the wall between two cork cells usually comprises five distinct layers. The innermost, on either side, is of cellulose; next the suberin lamella and between the two suberin lamellae is the middle lamella, which may be lignified or of pure cellulose. Where the wall is thin, the inner cellulose layer is missing.

In some of the material prepared for the present investigation, tabular cork was not found to be uniformly thickened. For example, in the species of *Quercus* taken, the radial walls of the cork were found in parts to be very much thinner than the tangential ones—the central region along the length of the radial walls being very thin (Figs. 1 and 2). Owing to growth in thickness of the stem, the periderm becomes stretched and frequently torn, and more generally it is at such lines of weakness that the layers split and become separated from each other, rather than along the line of the

middle lamella (Fig. 1). In this way the outer tangential wall of the inner cell adheres along the middle lamella to the inner tangential wall of the outer cell. These adhering walls, separated from the main mass of cork, may remain for some time attached edge to edge to similar walls, and in this way thin sheets of cork may become partly or wholly detached from the periderm tissue. It is this feature which is so useful in the present investigation of mechanical action.

Although several hundreds of sections of lichens growing on and in bark have been examined, no case was found of the perforation of a cork wall by lichen hyphae. This confirms the work of Bioret (2) and others.

The hyphae appear to force their way by pressure, due to growth, between the cell-walls; or it may be that they have the power to dissolve the middle lamella, and so separate the adjoining cork cells. Against the second alternative is the fact that there is no indication of chemical alteration of the walls in these regions, nor in fact in any region of the periderm where the lichen is attached.

That the corky tissue is not affected chemically by the presence of the lichen makes the study of the action of such plants a much simpler matter, for the effects of mechanical action only are indicated.

To determine whether there was any solvent action of the cork walls after inclusion in the lichen tissues, the thickness of included cork walls was compared with the walls of cells which were still in their normal state and position, and, generally speaking, the thickness was the same.

Frequently the outermost layers of periderm comprise empty cells, while the more deeply situated cork has cells which possess yellow or brown contents probably of the nature of tannins. The absence of such

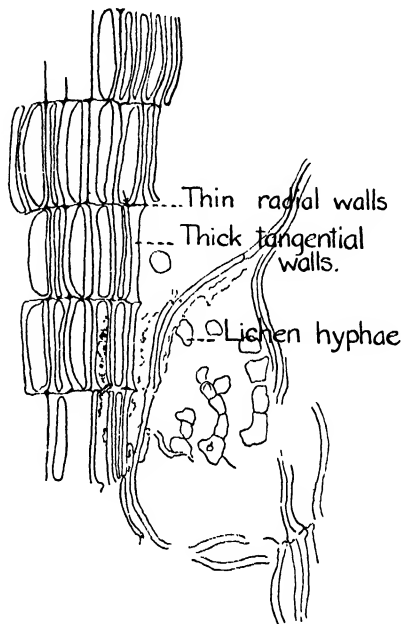


FIG. 1. Tangential section of tabular cork, showing thick tangential walls and thin radial walls.

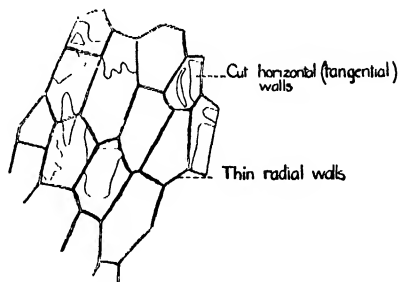


FIG. 2. Tangential section of cork, showing thin radial walls.

contents in the outer layers of cork does not appear to be due to the presence of the lichen, as has been formerly suggested, since there are numerous cases where these empty cells occur when lichens are not present, and also there are cases where the outer periderm cells possess such contents when the epiphloeodal lichens are present.

### III A. ACTION OF REPRODUCTIVE ORGANS ON THE SUBSTRATUM OF CORK.

Numerous sections of epiphloeodal and hypophloeodal lichens have been examined, and the most striking feature, which is common to all those with thick-walled tabular cork cells, is the remarkable arching of the periderm layers immediately below the fruiting bodies—whether these be of the lecanorine, lecideine, biatorine, or lirelline type (Figs. 3–13).

Fig. 3 illustrates a typical vertical section of a lecanorine apothecium growing on oak. Several layers of cork are arched under the apothecium and the dense tissue of the hypothecium ends abruptly above the arch. In the lower part of the hypothecium are several discontinuous layers of cork cells which have been separated from each other—either before or after the arching—by the growth of hyphae. The layer of periderm forming the concavity is continuous in this case, though frequently it becomes broken at the base of the arch and also in the curve of the arch itself. Immediately below the position where the arch of cork layers joins the horizontal tissue of the tree there is frequently a separation of further periderm layers (Figs. 3 and 4). Enclosed in the concavity there are very few hyphae. These increase in number when the arch of periderm becomes ruptured, for the hyphae, growing from the hypothecium, crowd into the space (Fig. 4).

Even in the most crowded ‘apothecial arch’ observed, the number of hyphae was insufficient to have caused the elevation of the periderm in the manner described.

Another important fact in this connexion is that the position of the enclosed hyphae indicate that their growth in these arches is subsequent to the formation of the arch (Figs. 3, 4, and 5). They appear to grow up from the bottom of the cavity, down from the apex and out from the walls. It may be that in some cases the hyphal elements were present between the layers of horizontal cork cells before the periderm became arched beneath the reproductive organ, and these elements may be the origin of the hyphae present in the later stages when the arch remains intact.

Sometimes the raised cork layers bordering the hollows are separated by dense hyphal tissue (Fig. 5). Reference to this will be made later in connexion with the action of the thallus.

A series of horizontal sections through the thallus in the apothecial

regions is particularly striking, and shows very clearly the circular shape of the 'apothecial arch' in horizontal section (Figs. 6, 7, and 8).

That the 'reproductive arch' is not always of the rounded shape is shown by that which occurs below a lecanorine apothecium of a lichen of

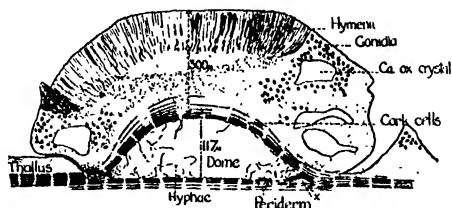


FIG. 3. (Semi-diagrammatic.) Vertical section of a lecanorine apothecium on oak, showing arching of periderm cells below fruiting body. Note extra pull at 'x'.

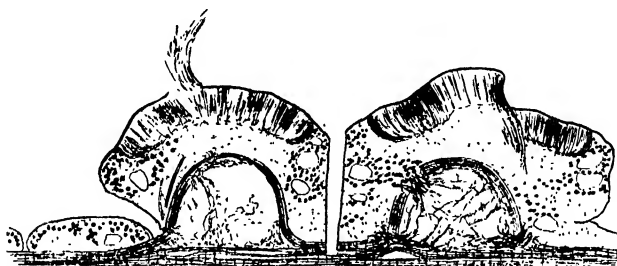


FIG. 4. (Semi-diagrammatic.) Vertical section through two apothecia, showing arches or 'domes' of periderm which have been broken, and through the gaps of which hyphae from the surrounding tissues crowd.

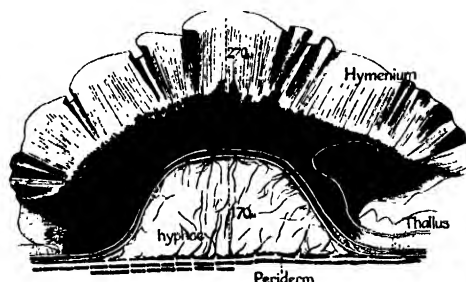


FIG. 5. (Semi-diagrammatic.) Vertical section of lecideine apothecium, showing arching of the periderm. Note puckering of hymenium.

loose or pulverulent texture (Fig. 9). The fact that the tissue below the hymenium in this case is of a looser texture than the densely packed hyphae of the lecanorine and lecideine types previously quoted may account partly for the difference. The nature of the cork, too, may be an important factor.

Although in the Graphidineae we are dealing with reproductive bodies of a very different form and structure from the previous examples, yet we find the action of the lirellae on a thick-walled cork substratum is practically the same—the periderm showing the usual arching under the fruiting organs. It will be noticed that in the case of the younger lirellae

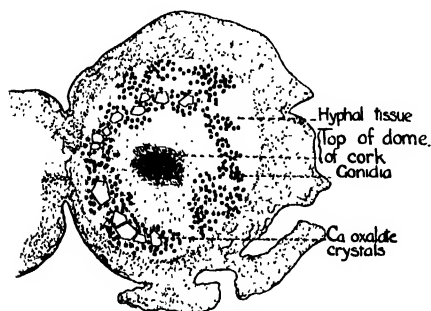


FIG. 6. (Semi-diagrammatic.) Horizontal section of lecanorine apothecium on bark, showing top of 'dome' of cork cells.

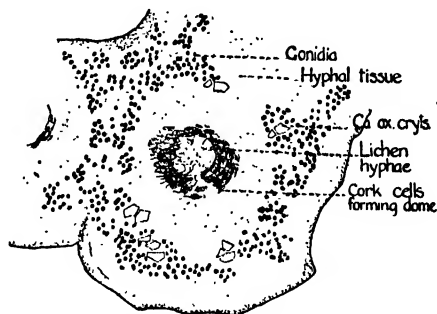


FIG. 7. (Semi-diagrammatic.) Horizontal section of lecanorine apothecium on bark, showing 'dome' of periderm with top cut off.

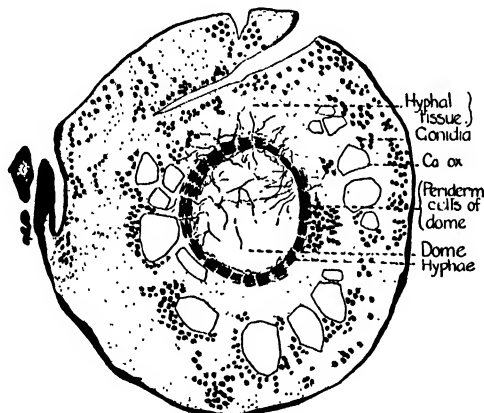


FIG. 8. (Semi-diagrammatic.) Horizontal section of lecanorine apothecium on bark, cut near the base of the 'dome', showing the cavity with a few hyphae penetrating from surrounding tissues.

(Fig. 10)—i.e. those with a single carbonaceous wall—that there are very few, if any, hyphae within the dome of cork; whereas in the older lirellae (Figs. 11 and 12) there is considerably more hyphal tissue which sometimes gives the appearance, in fresh hand-cut sections, of a gelatinous mass. This was noticed by Wolff (9), who suggested that it was probably of the nature of a food reserve beneath the fruit body. The present writer suggests that—as in the previous examples quoted—it is merely the ingrowth of the ordinary hypothecial hyphae into the cavity below the lirellae.

It is important to notice that the position of the greatest arching of the cork is immediately below the uncarbonized part of the floor of the

rejuvenated lirella, where the cork is more in contact with the gelatinous tissues of the reproductive organ (Fig. 12).

That these upheavals of the horizontal layers are not peculiar to the apothecial regions will be shown in the discussion of the thallus action, and that each arching is not the peculiar action of a single apothecium is shown

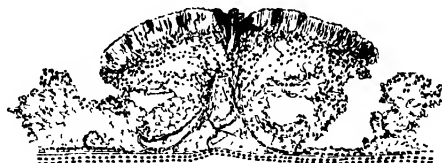


FIG. 9. Vertical section through lecanorine apothecium of pulverulent lichen, showing arching of the cork walls. Note the absence of cortex and loose texture of the thallus.



FIG. 10. (Semi-diagrammatic.) Vertical section through young lirellae of *Graphis elegans*, showing absence of hyphae in 'dome' of periderm below A; a few below B.



FIG. 11. (Semi-diagrammatic.) Vertical section through lirella of *Graphis elegans*. In the 'dome' there appears to be a mass of gelatinous material 'X', but under high power this proves to be a dense web of hyphae.

by the fact that whenever two or even more apothecia have arisen close together, then one large arch of cork is formed, showing that whatever is the cause of the action, that cause is more effective when two or more apothecia are in such a position as to have a combined action on the substratum (Fig. 13).

The nature of this action is discussed in the next section, but before passing on to it, a particularly instructive effect, produced when certain species of the Graphidineae grow on soft or thin-walled cork—such as Ash—may be cited (Fig. 14). In the region below the thallus tissues,

and in amongst which the hyphae branch, the cells appear crushed and misshapen, but below the lirellae the cells are drawn out, or elongated at

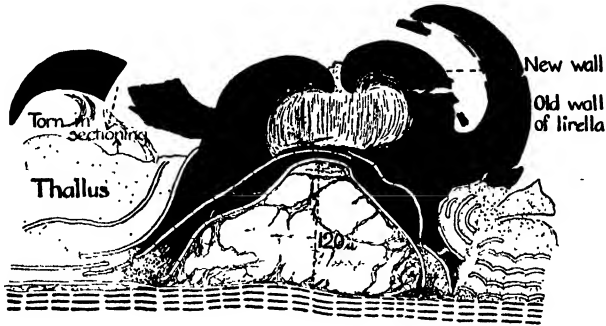


FIG. 12. (Semi-diagrammatic.) Vertical section through lirella of *Graphis elegans*, showing doming beneath fruiting organ. Note that greatest height of the arch is immediately below uncarbonized part of floor.

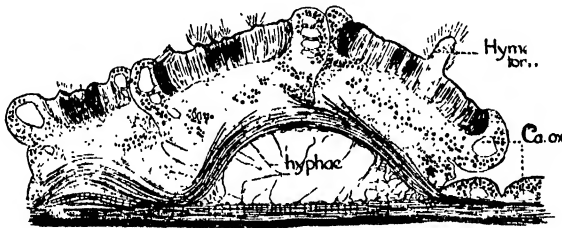


FIG. 13. (Semi-diagrammatic.) Vertical section through 'reproductive arch' formed by two lecanorine apothecium. Note puckering and tearing of gelatinous hymenial tissue.

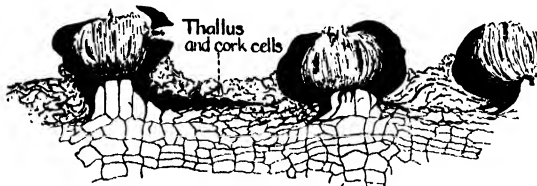


FIG. 14. (Semi-diagrammatic.) Vertical section through hypophloeodal lichen on thin-walled cork. Note that below the lirellae the thin-walled cork cells are drawn out at right angles to the surface.

right angles to the surface of the bark, seeming to indicate that there is a pull at right angles to the surface in these regions.

### III B. (i) *Discussion of the Action of the Reproductive Organs on the Substratum.*

It is useful at this stage to consider the cause of the elevation of the superficial layers of periderm below the reproductive bodies since it helps to elucidate the problem of the thallus action.



The following are the relevant facts:—

- (1) the arching is strictly limited to the region immediately below the fruiting bodies;
- (2) the arch is larger when two or three apothecia have arisen close together;
- (3) the arching follows in the periderm the lines of the lirella above;
- (4) the arching is obviously not due to the growth of the few hyphae present in the cavities.

These all go to show that the *form* of the reproductive organs is one of the

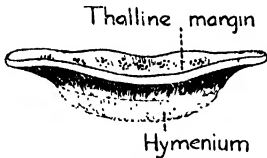


FIG. 15. (Diagrammatic.) Horizontal section of lecanorine apothecium mounted in water. Note swelling of hymenium.

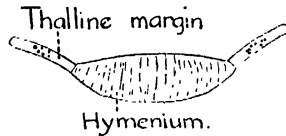


FIG. 16. (Diagrammatic.) Section through Fig. 15 showing hymenium very swollen.



FIG. 17. (Diagrammatic.) See text.



FIG. 18. (Diagrammatic.) See text.

chief factors in the production of this phenomenon. That which is common to all the fruiting bodies and which follows out the form of these organs is the hymenial tissue. Also, when two or more apothecia are close together, the hymenial tissue of the group is correspondingly increased in area.

Since the hymenium is never actually in contact with the periderm—although in *Graphis elegans* (Fig. 12) it is very close to it—it is not necessary to look for a chemical cause (the term 'chemical' being used in the strict sense) as being the explanation of the phenomenon. There is then left only a physical or mechanical action to account for the effect produced.

The hymenium in all lichen fruits is gelatinous and readily absorbs water. During this process it expands enormously. The folding or puckering up of the hymenial layers, shown in Figs. 3, 4, 5, 9, and 13, is an indication of this, but one has only to cut a section of an apothecium and mount it in water to understand the enormous capacity for absorption the hymenium possesses. For example, a horizontal section of a lecanorine apothecium, when mounted in water, shows almost immediately the hymenium pushing up or down out of the original plane of the section, and a distortion of the thalline margin (Figs. 15 and 16). A longitudinal section, when mounted in water, also shows this expansion of the hymenium (Figs. 17, 18, and 19).

When a whole apothecium is detached from the substratum it leaves exposed a small, apparently bare, circular area of cork, surrounded by the torn sides of the thallus. If the detached apothecium be inverted, the lower side is seen to resemble a small shallow cup lined with smooth cork. In the cavity of the hollow one finds a few white hyphae attached to some part or other of the wall. When water is allowed to come in contact with the hymenium layer and the lower side of the apothecium, with its layers of adhering cork, is watched, the cup-like hollow, which at first was more or less shallow, seems gradually to deepen, indicating that the curve of the arch of cork has become greater as a result of the addition of water to the gelatinous hymenial tissue.

The swelling power of the hymenial gelatin of the Graphidineae can

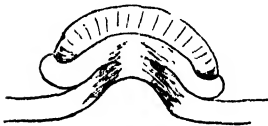


FIG. 19. (Diagrammatic.)  
See text.



FIG. 20. (Diagrammatic.)  
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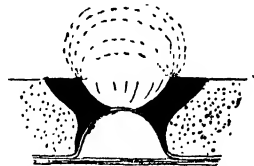


FIG. 21. (Diagrammatic.)  
See text.

be strikingly illustrated by shaving off with a razor the parts of the lirellae jutting out above the general level of the surface of the bark, and then wetting with a soft brush the surface of the lichen. Immediately the positions of the lirellae are indicated by gelatinous worm-like excrescences standing out above the level of the bark (Figs. 20 and 21). When this gelatinous mass is confined within the hard carbonaceous walls of the lirella, the latter, on addition of water, are pushed and pressed back to allow of this expansion. The natural result on the substratum of this action is indicated by Fig. 14. The less resistant periderm layers become pulled or levered up by the outward and downward movements of the lirella walls.

It is a similar kind of action which goes on in the region of the apothecium. In this case the thalline or proper margins form the more resistant tissue. This is one with the surrounding thallus which is firmly fixed to the substratum. The margins are pressed backwards and downwards by the swelling gelatinous hymenium. This brings about a certain amount of the arching of the substratum, though it is not entirely responsible for it. One concludes that as the gelatinous hymenium increases in size, so its action on the substratum increases. The arching is continually repeated, but after each action the reproductive organ never goes back exactly to its previous position, for it is during such periods of turgescence that growth is more vigorous.

(ii) *Experiments with Gelatin and Rubber.*

In order to demonstrate that the gelatinous tissues are responsible for such action described, models of apothecia of different sizes were cut out of a 20 per cent. gelatin gel. These were then made to adhere to a substratum of thin stretched rubber by smearing the upper surface of the latter all over with the same gelatin sol. (Thin sheets of paraffin wax and cuticle were also tried as substrata, but for the purpose in hand the rubber of toy balloons was found the most efficient.) The rubber was stretched over table-napkin rings and tied down round the edge. In this way the lower side of the substratum could be observed without difficulty. The whole upper surface was smeared with gelatin, so that the artificial apothecia were the only parts which were not common to the whole surface. The gelatin

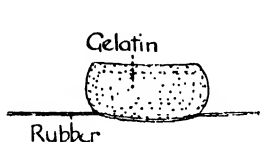


FIG. 22. (Diagrammatic.) See text.

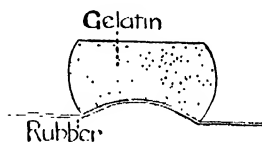


FIG. 23. (Diagrammatic.) See text.

was allowed to set and dry in the laboratory for a few hours. After this period, examination of the lower surface of the stretched rubber showed that the parts below the artificial apothecia had become convex (Fig. 22). This was due to evaporation from the exposed upper side of the artificial apothecium having taken place, but there was little or no loss of water from the lower part, which was protected both by the mass of gelatin above and by the rubber below. The natural result of this evaporation is the slight expansion of the lower side of the gelatin. After immersion in water for a few minutes the convexity of the rubber on the lower side vanished. In a few more minutes the rubber in these positions was seen to arch. On drying the gelatin the rubber resumed its natural position. After continued immersion in water for one and a half to two hours, the arching of the rubber below the artificial apothecia was very pronounced (Fig. 23). This again vanished on evaporation of the water under laboratory conditions. Addition of a small amount of dilute hydrochloric acid to the water in which the experiments were immersed caused these concavities of the rubber to become very much larger, and even the gelatin film had the power to pull up the rubber to a slight extent.

The arching of the substratum of rubber is caused by the gelatin expanding on absorption of water, and since the gelatin adheres to the rubber it causes this to expand with it. At first, however, the distribution of the water in the gelatin is unequal, since it is absorbed at the upper surface and sides only. These expand more rapidly than the lower regions,

and since there is cohesion between all the particles in the mass, they pull up, on expansion, the lower regions with them. These lower zones of gelatin adhere to the substratum of rubber, which therefore in its turn is pulled up by the gelatin. That this arch becomes more pronounced and larger as the absorption of water increases is due to the fact that in time the lower regions of gelatin take up more water and expand, causing the rubber substratum to expand with them. The thinner films have less action than the thicker masses of gelatin representing the apothecia—the expansion of the rubber being less on account of the smaller absorption by the superficial gelatin. This fact is important in connexion with the greater action of apothecia compared with the lesser action of thalli on substrata.

As is well known small amounts of acid causes gels to absorb water to a very much greater extent. This is important, for when dealing with living lichen tissues we have to consider the carbon dioxide of respiration, and the evolution of this gas is more vigorous in more actively growing regions such as the reproductive organs.

The fact that gelatin, when not enclosed on three sides by walls of more resistant material, as in lirellae and apothecia, can produce an arching of the substratum on addition of water, goes to show that the suggestion already given for the arching of the periderm by the hymenium is not the full explanation of this phenomenon. The expansion of the lower layers of gelatin in contact with the substratum causes the latter to expand and arch up in its attempt to obtain more room.

### (iii) *Experiments with Reproductive Bodies on a Rubber Substratum.*

A second series of experiments was carried out, using actual apothecia and lirellae in the place of artificial ones—the rubber and film of gelatin being prepared as before. Apothecia of saxicolous lichens as well as bark species were used, large and small examples being taken. The lirellae of certain members of the Graphidineae were also selected.

The apothecia were pulled off carefully with a pair of forceps, and the lower irregular parts cut off plane with a razor. As previously described for artificial apothecia the reproductive organs were then made to adhere to the rubber. The lirellae were removed whole and stuck on to the gelatin film.

In a large number of cases, after the experiments had been left to set and dry, there was a tendency of the substratum to arch below the apothecia and lirellae. This can be explained by the absorption of water from the gelatin immediately below the apothecia by the less saturated lichen substance, the apothecia being detached when dry. The apothecia would therefore expand very slightly causing the arching observed. On immersion of the rubber substratum with its attached apothecia and lirellae for about

ten minutes, there were distinct concavities formed in the substratum below the reproductive bodies. Below the apothecia the arches were very small, but were quite distinct. Below the larger apothecia correspondingly larger arches were present, and below the lirellae grooves, following out the line of the reproductive bodies on the upper surface, were formed. After half an hour's immersion these concavities became more pronounced. It was noticed that the lirellae responded more readily than did the apothecia, taking only five minutes to show an arching of the rubber, while a few of the softer apothecia took little longer to act on the substratum. Apothecia of a somewhat hard nature, such as those of *Lecanora parella*, required about twenty minutes before responding definitely. This arching in the case of *Lecanora parella* became more pronounced when the water was allowed to percolate through the tissues. Other experiments showed that certain species of *Graphis* could respond in two minutes, while species of *Lecanora* took ten to fifteen.

There seems little room for doubt that the arching of the substratum below the reproductive organs is due to the absorption of water by the gelatinous tissues of these bodies. But the cork is not so elastic as rubber, and consequently frequent breaks occur in the raised cork layers (see Figs. 4, 5, 9, 10, and 12).

The gelatin retained a strong hold on the substratum of rubber even when the former contained a fair amount of water. This hold became less as the gel became saturated, so that frequently the over-soaked artificial apothecia were very easily detached. The same holds for apothecia attached in a similar manner—the gelatin being the cause of detachment in these cases also. It has been found by experiments in maceration, and also from observation that it takes a very long time for water to soak through an apothecium and saturate the hyphae attaching the fruiting body to the substratum. Any pull from the swelling superficial tissues is conveyed by the lower tissues to the substratum, and either the substratum must respond by tearing its superficial layers free from the more deeply seated part, as in tabular cork, or as in certain rocks the attaching hyphae must break free of the substratum.

#### IV A. ACTION OF THE THALLUS ON A CORK SUBSTRATUM.

The wedge-like mechanical action which is common to all lichens, and is due to the growth of the hyphae, is possessed by the hypophloeodal lichens in a marked degree. It has been previously described by Miss Mellor (7), Bioret (2), and the present writer (5). The hyphae at the growing periphery of the lichen often force their way between the cells of the periderm. The layers of cork thus become incorporated in the lichen thallus or raised entirely above it (Bioret, loc. cit.) (Fig. 24). As develop-

ment proceeds so the cork layers become raised further from their original position in the same way as that described for the lifting of shale plates in *Lecidea lucida* (5). When an apothecium develops in the tissues above such raised layers of cork the arching below the fruiting body appears to be the result of a more complicated action, but this is not really the case.

Owing to the method of renewal of lichen tissues by the wearing away and replacement of the superficial layers (8), the periderm cells, which ultimately rest on the surface of the thallus, in time come to be removed either in patches or as a whole. Should the periderm covering be a fairly

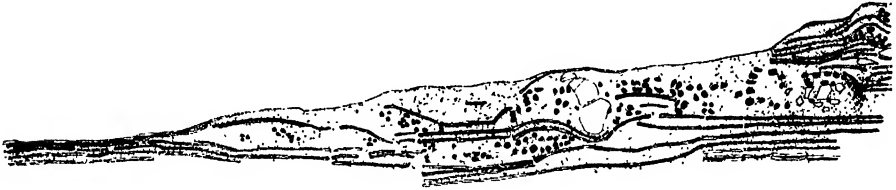


FIG. 24. (Diagrammatic.) Section through the peripheral growing region of a bark lichen, showing the wedge action of the hyphae pushing apart the cork walls.

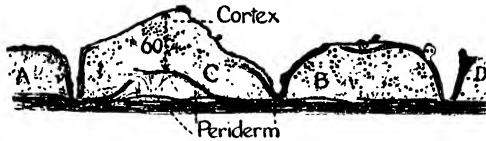


FIG. 25. (Semi-diagrammatic.) Vertical section of hypophloeodal lichen, showing position of superficial cork layers and position of arching. Note that arching comes below region free of superficial cork; also presence of cortex in this region.

constant feature of the lichen then the species is a hypophloeodal one, but should the lifted cork layers be only a temporary feature of the plant then the species is an epiphloeodal form. In some species of hypophloeodal lichens, such as that shown in Fig. 25, the thallus is massed below three or four layers of tabular periderm walls. The thallus is of the ordinary type but with this one difference, that where the periderm layers cover the thallus as at A and B in Fig. 25 there is no cortical tissue formed, the cork resting directly on the gonidial layer. Where the layers have been removed from their protective position by weathering a cortex is formed (Fig. 25, C). This fact throws some light on the main function of the cortical tissue of lichens, namely, that it is a protective layer since the presence of the cork cells make the formation of cortex unnecessary. It explains the thickness of the cortex in many of the epiphloeodal lichens which grow in exposed positions.

In these hypophloeodal species lifting or arching of the cork cells below the thallus is rather uncommon, though not unknown. It occurs in those parts of the thallus from which the superficial periderm layers have

been removed (Fig. 25, c) but not below every such part. The arching is of a type similar to that previously described for the action of reproductive bodies, and is obviously not the result of the wedge-like mechanical action (loc. cit.). The superficial periderm layers do not extend without a break over a number of areolae, but are frequently broken at the peripheries of the areolae (Fig. 25, A, B, D).

A comparison of the thickness of areolae above such arches with that of areolae below which there is no elevation of the periderm layers shows

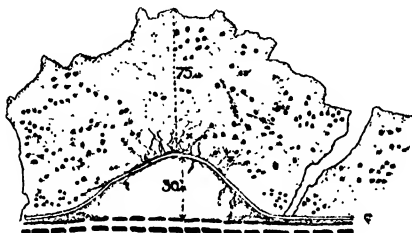


FIG. 26. (Semi-diagrammatic.) Vertical section of areola of an epiphloeodal lichen, showing 'doming' of periderm walls. The dome contains very few hyphae. Note that at 'x', the centre of the areola, next to the raised cork layer, the hyphae form a loose network only.

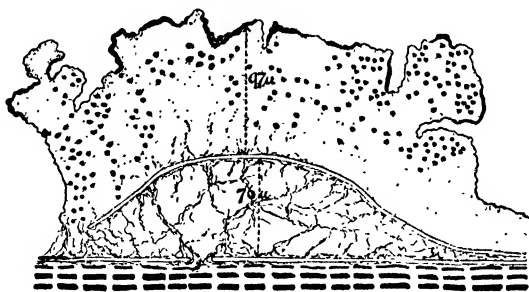


FIG. 27. (Semi-diagrammatic.) Vertical section of an areola of an epiphloeodal lichen, showing 'dome' of cork walls with many hyphae beneath it, but not forming a dense tissue.

that the arching ('doming') is not correlated with the thickness of the thallus. In the example illustrated in Fig. 25 the thickness of the thallus was  $60\mu$ , while hypophloeodal areolae, below which there occurred no arching, were  $82\mu$ ,  $105\mu$ , and even  $120\mu$  thick.

The hypophloeodal species which grow in thin-walled cork do not possess definite gonidial, cortical, and medullary tissues, but form a loose network of hyphae and gonidia among the cork cells, the latter, as a result, becoming somewhat crushed.

In epiphloeodal lichens, even when the thallus is no thicker than that of the hypophloeodal forms, arching, similar to that found below reproductive bodies, is common (Figs. 26 and 27). In some cases the height of elevation is not much less than the thickness of the thallus above it. In

the epiphloeodal forms the raised cork layers often become broken at the periphery of the areolae (Figs. 26, 27, and 28) like the superficial periderm of the hypophloeodal species. In time these arches of periderm become filled with ingrowing hyphae from the surrounding tissue, the tissue becoming denser, and the cork appearing as if raised by the mass of hyphae below, i. e. as if formed by the 'mechanical wedge' action (Fig. 29). This action may occur in these lichens, but it can readily be detected, even in the early stages, by the dense growth of tissue below such raised cork.

It has been mentioned several times in connexion with the thallus

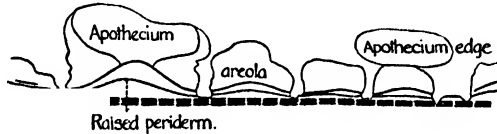


FIG. 28. (Semi-diagrammatic.) Vertical section of lichen, showing raised cork layers broken at the periphery of each areola. Compare height of arch below apothecium with that below areola.

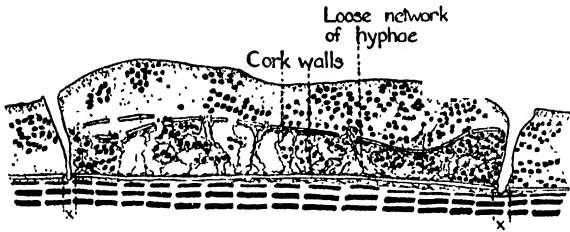


FIG. 29. (Semi-diagrammatic.) Vertical section of thallus of epiphloeodal lichen. The raised periderm layer has both hyphae and gonidia beneath it, but the hyphae form a loose network only. Note that the lower detached layer of cork walls is broken at the periphery of the areola at 'x'.

action that the raised periderm layers are rather discontinuous, being broken at the periphery of the areolae (Figs. 26, 27, and 28). This indicates that the greatest strain on the cork occurs in the layers below, or in the lower part of the peripheral regions of the areolae. Towards the centre, in the lower part of the areolae, the lichen tissue is often looser in texture than in the more peripheral regions.

It has been shown by a comparison of hypophloeodal and epiphloeodal forms that thickness of thallus is not correlated with the arching of the cork below the areolae. The fact that the superficial impermeable periderm prevents the arching of the substratum below the thallus seems to indicate that, as with the reproductive bodies, the cause of the elevation of the periderm is, probably, the swelling of the gelatinous material of the thallus tissue. These tissues are not nearly so gelatinous as the hymenia of fruiting organs, but a microscopic examination of the lichen hyphae shows there to be a considerable amount of this substance making up the structure of the thallus. As will be seen later, the effect of a dense weft of



such hyphae when moistened with water is similar to, though not so great as, that of a gelatinous mass under the same conditions. This, together with the small size and thinness of the areolae as compared with the apothecia, accounts for the small size of the arches produced below the thallus as compared with those formed below the apothecia.

Another illustration of the fact that it is the nature and not the mere thickness of the thallus which is the most important factor in producing the

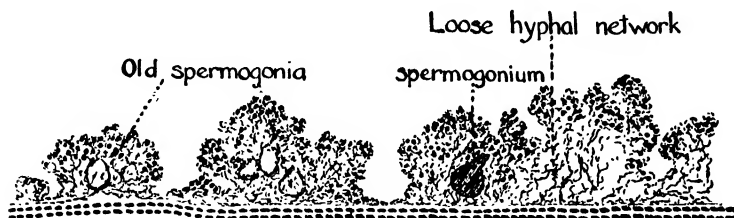


FIG. 30. (Semi-diagrammatic.) Section through epiphloeodal, pulverulent lichen, showing absence of arching of the cork substratum.

arching of the cork is that there is no arching whatever below the ordinary thallus of a pulverulent or powdery lichen (Figs. 9 and 30). The hyphae form a very loose network, and the gonidia, wound round by hyphae, lie exposed on the irregular surface. There is no definite cohesion between the hyphae of the thallus—no definite tissue—as in other epiphloeodal forms. Although such thalli may reach a thickness of  $440\ \mu$  or more, compared with  $100\ \mu$  of the previous types, yet they are unable to arch the periderm substratum.

#### IV B. DISCUSSION OF THE THALLUS ACTION.

##### (i) *Experiments with Gelatin and Rubber.*

As in the case of the investigation of the mechanical action of the reproductive organs on the cork, stretched rubber was used for the substratum of which small areas were covered with a 20 per cent. gelatin sol. When the gelatin had set some of the films were cut down to the rubber substratum in such a way as to represent the 'chinking' or cracking of a typical crustaceous lichen (Fig. 31). Other patches of gelatin were left uncut. Some of the cut and uncut patches were covered over completely with a layer of stretched rubber, which was tied down at the rim of the ring over which the substratum rubber was also tied. In this way both evaporation and absorption of water were prevented. Other small cut and uncut patches of gelatin were covered over less completely with superficial rubber, i. e. the patches of gelatin were just covered with rubber of the same area as themselves, so that it was possible for evaporation and absorption of water to take place at the peripheral regions of the gelatin (Fig. 32). These

represented the cases of the hypophloeodal species where the periderm layers had broken at the periphery of the areolae. All were then set to dry for about half an hour under room conditions. At the end of this period the following effects on the substratum were noticed when the latter was examined from the underside:

1. The rubber below the non-cut gelatin films was convex owing to evaporation of water from, and consequent contraction of, the superficial gelatin layers.

2. The rubber below the 'chinked' films was expanded downwards as a whole, but very little expansion—or none at all—occurred below the actual cuts in the gelatin above. The cuts were therefore indicated below as grooves in the rubber surface (Fig. 33). This effect was due to evaporation from the surface of each of the artificial 'areolae' and a consequent

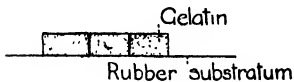


FIG. 31. See text.

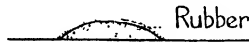


FIG. 32. See text.



FIG. 33. See text.

contraction of the surface layers. This contraction both in the cut and uncut films resulted in an expansion of the lower zones adhering to the rubber, and consequently a stretching of the rubber took place. This expansion in the uncut film was indicated by one large convexity of the rubber substratum, and in the cut films by as many convexities as there were artificial areolae in the film. The cuts or 'chinks' in the gelatin were represented as grooves, since they were the limits of the convexities.

3. The lower surface of the rubber was unaltered below the completely covered 'chinked' and 'unchinked' films.

4. The rubber below the less well-covered cut and uncut gelatin films showed only an indication of the effect produced by the uncovered 'chinked' and 'unchinked' gelatin films.

On examination of the lower side of the substratum after immersion in water for five minutes, the following effects were noticed:

(1) The rubber below the uncut films was arched as a whole.

(2) The rubber below the cut films was arched as a whole, but the 'chinks' in the gelatin were represented by sharp ridges (Fig. 34). It was noticed that the surface of the 'areolae' did not regain their plane form, nor become convex on absorption of water, but remained slightly concave.

(3) There was no alteration in the substratum below the patches of gelatin completely covered with rubber.

(4) There were indications only of a general concavity in the lower surface of the rubber below the patches of gelatin incompletely covered

with rubber. The cuts in the cut patches similarly treated were only very slightly indicated even after ten minutes immersion.

After one and a half to two hours immersion the effects in (1) and (2) were increased slightly, but in (3) and (4) there was practically no alteration. It was noticed that a long immersion in water—one hour—caused the ridges below the 'chinks' to become less sharply marked (Fig. 35), and that on longer immersion they disappeared altogether.

It is seen that the presence of a substance above the gelatin film, hindering absorption and evaporation, prevents the reaction on the substratum. From this one can conclude that the presence of the superficial periderm on the hypophloeodal lichen type described was responsible for the absence of arching in the cork layers below.

The effect of arching under the artificial areolae and the ridging of the

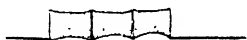


FIG. 34. See text.



FIG. 35. See text.

rubber below the cuts is an exactly similar effect to that noticed in the periderm below the crustaceous bark lichens. It is the parts of the rubber below the cuts in the gelatin films which seem to suffer the greatest strain, and in this connexion it is interesting to recall that the periderm layers are generally broken in the regions of the 'chinks' in corticolous lichen thalli.

#### (ii) *Experiments with actual Thalli on a Substratum of Rubber.*

Actual thalli were made to adhere to a rubber substratum in the same manner as described for the adherence of the apothecia. *Lecanora parella* and *Aspicilia calcarea* were used, but of the latter one could obtain larger patches, and so it was preferred. The thalli of *Aspicilia* were obtained clear of the limestone substratum by decalcification with dilute hydrochloric acid, thoroughly washed in water and allowed to dry under laboratory conditions. In the samples used it was noticed that there were practically no rhizoidal hyphae of the usual type (1 and 4). There were, however, numerous small clumps of the swollen oil-cells (4) which projected a short way from the lower surface. After the thallus, bearing numerous apothecia, had been allowed to stand for some time while the film of gelatin set, it was noticed that the rubber below the thallus as a whole had become convex. The separate areolae of the thallus were too small to be able to affect individually the comparatively thick rubber substratum. The experiment was then immersed in water for one minute, after which there was an arching of the substratum of the whole area of rubber covered with the thallus. After another four minutes this was more pronounced. Below

the larger cracks across the thallus there were ridges on the lower side of the rubber, just as below the cuts in the gelatin films there were ridges on the substratum. The positions of the larger apothecia and groups of apothecia in the thallus were indicated on the lower side of the rubber by very distinct concavities within the general arch below the thallus.

This result brings out very clearly the fact that, though both apothecia and thalli have a mechanical action on the substratum, the greater effect is caused by the former. It also throws light on a phenomenon often noticed by the present writer—that when fruiting bodies of crustaceous lichens are detached from a shale substratum there are generally small flakes of the substratum attached to their lower surface. It is also true in some cases of the thallus, but to a much less extent. This was clearly illustrated during some attempts to peel off crustaceous lichens from a shale substratum by drying gelatin upon their surfaces. The lichens came away with the gelatin, leaving in some places the lower part of their thalli attached to the substratum, while in other places shale fragments were pulled away with the thallus. But below the fruiting bodies generally there were attached shale plates. Miss Mellor (7) also stated that she has noticed in lichens growing on glass that scales of glass are found most frequently in the apothecial and spermogonial parts of the thallus; she observed the same effect for *Squamaria saxicola* on slate. It was not until the effect of apothecia on a periderm substratum was first seen that a possible explanation of this phenomenon suggested itself.

From the foregoing observations and simple experiments it has been shown that the action of corticolous lichens on their substratum is mechanical and not chemical, and that this mechanical action is brought about by the expansion and contraction of the lichen tissues on absorption and loss of water.

#### SUMMARY.

1. In corticolous lichens the superficial layers of periderm are arched below the reproductive bodies. There is apparently no chemical alteration of the cork.

2. Experiments with artificial reproductive bodies made of gelatin show that, on absorption of water by the latter, the arching effect can be produced on a substratum of stretched rubber.

3. Experiments with actual reproductive bodies, made to adhere to a substratum of stretched rubber, show that on absorption of water by the lichen tissues the rubber becomes arched below these organs.

4. The arching is caused by the swelling of the gelatinous tissues of the fruiting organs and is the result of a mechanical action.

5. In certain corticolous lichens elevation of the periderm layers occurs

below the areolae, and is shown to be correlated with the nature and not with the thickness of the thallus.

6. Experiments with gelatin, made to represent thalli of lichens and placed on a stretched rubber substratum, show that the elevation of the latter is due to the absorption of water by the gelatin.

7. Absorption of water by actual thalli placed on a rubber substratum causes an arching of the rubber.

8. The effect of the thallus on the substratum is due to the absorption and loss of water by the lichen tissues, and is therefore the result of mechanical action.

9. Such mechanical action is greater below the reproductive bodies than below the ordinary tissues of the thallus.

The writer wishes to thank Sir Frederick Keeble, Sherardian Professor of Botany, Oxford, for his kindness in providing equipment and facilities for work during this and other investigations.

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# Floral Construction in the Helobiales.

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With eight Figures in the Text.

THE studies previously published by the writer on the floral construction of various members of the Ranunculaceae have shown that the flower of this family is probably derived from a trimerous type, though this is often obscured by fission and fusion of parts, or even by abortion (cf. Salisbury, 'Ann. Bot.', 1919 and 1920). In view of the results obtained it seemed desirable to ascertain what variation normally occurs in flowers of Monocotyledons where the trimerous construction is the rule, instead of being exceptional or obscured as in most Dicotyledons. For such a comparison the Helobiales and particularly the Alismataceae were clearly indicated, not merely on account of a possible relationship, but because of the numerous parts in the androecium and gynoecium which this family exhibits.

The chief species studied were *Alisma plantago*, *Echinodorus ranunculoides*, *Butomus umbellatus*, and *Sagittaria sagittifolia*. For assistance in obtaining flowering material of the last-named I am indebted to Mr. V. S. Summerhayes. Of these four species sufficient material was available to enable the meristic variation to be studied.

Of *Sagittaria obtusa*, *Stratiotes aloides*, and *Hydrocharis morsus-ranae* only a comparatively small number of flowers were available: too few to justify any statistical conclusions.

## (A) ALISMA PLANTAGO, L.

This species was studied in a number of different localities. Both the type and the variety *lanceolatum* were examined, but the two do not appear to exhibit any essential differences in floral construction.

As is well known, the flower possesses a perianth of two trimerous whorls, the outer being green and small, the inner pink and much larger.

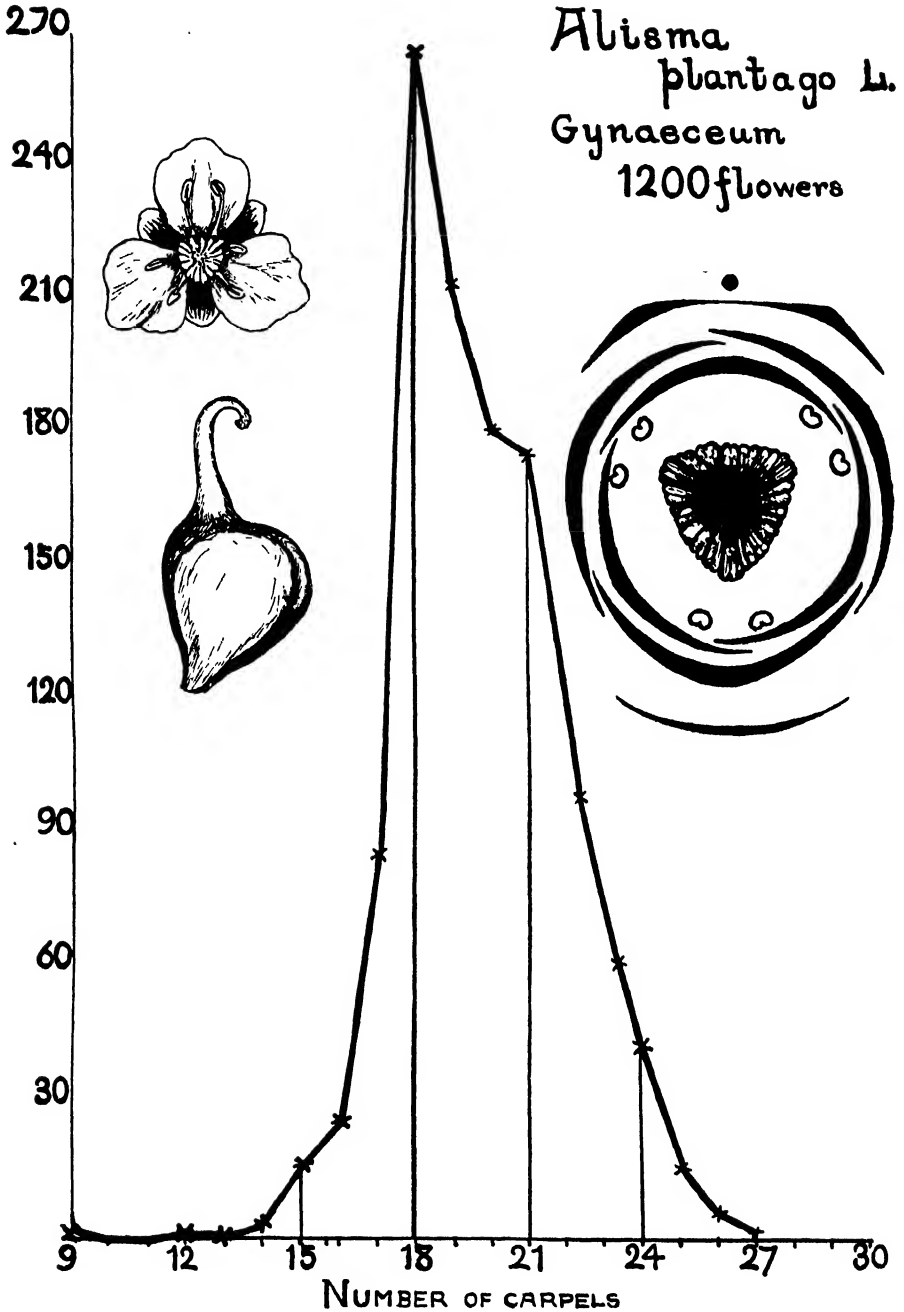


FIG. 1. Meristic variation in the gynaeceum of 1,200 flowers of *Alisma plantago*, showing a primary mode at 18 and a secondary mode at 21. On the right is shown a floral diagram of a typical flower, and on the left a flower seen from above and a single carpel from the side. Ordinates represent the number of examples and abscissae the number of carpels.



The stamens are six in number in a single whorl, whilst the carpels vary considerably in number, but also appear to be in a single whorl (cf. Fig. 1). It is of interest to note that the membranous brown scale leaves borne upon the inflorescence axis occur in alternating whorls of three members each, but the vegetative leaves exhibit the two-fifths phyllotaxy so common amongst Dicotyledons.

Despite the large number of flowers examined, only one instance of variation in the androecium was encountered.

The meristic variation of the gynaecium was studied in six plants,

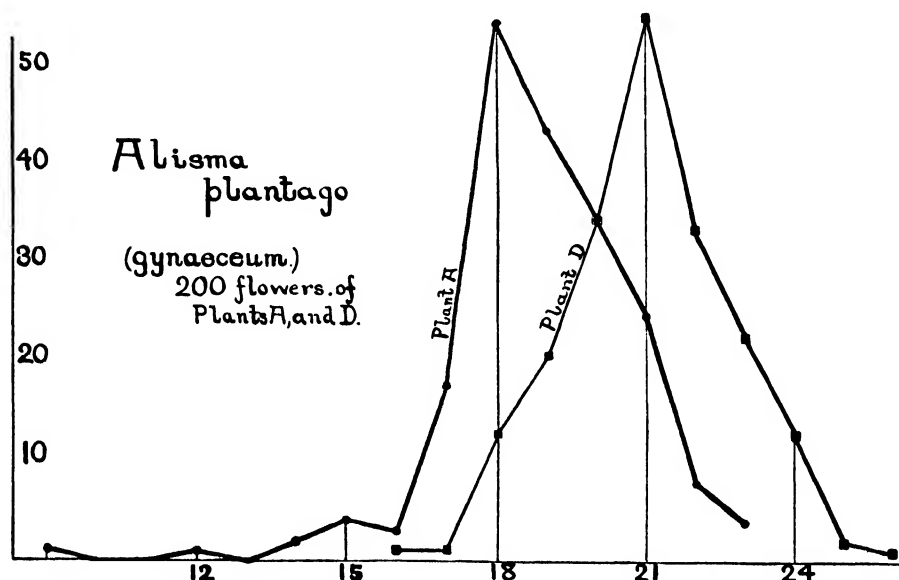


FIG. 2. Meristic variation in the gynaecium of two separate plants of *A. plantago* (200 in each case), showing modes at 18 and 21 respectively. In the 'curve' for plant 'A' the occurrence of secondary modes is well seen.

200 flowers being examined in each case. The data are presented in Table I and in Figs. 1 and 2.

From these data several important facts can be recognized. The 'curve' of meristic variation in Fig. 1 for the whole 1,200 flowers shows that even in the stereotyped Monocotyledonous flower the tendency to vary is quite evident when the number of parts involved is comparatively large. Moreover, we note that, whilst there is a primary mode at 18, there is a decided secondary mode at 21 and a less conspicuous one at 15. The 'curves' for individual plants shown in Fig. 2 bring out the fact that the primary mode may vary with the individual, thus plant A has the mode at 18 and plant B at 21. Plants E and F (cf. Table I) agree with plant A, whilst plant D agrees with B. Plant C, however, is peculiar in having the mode at a number which is not a multiple of three (cf. Table I)

Secondary modes may, however, be quite pronounced in the case of a single individual, as is seen from the 'curve' for plant A, Fig. 2, where secondary modes are indicated at 15 and 21.

Amongst the Ranunculaceae, perhaps the most striking feature of the meristic variation in the androecium of *Eranthis hyemalis*, *Ficaria verna*, *Anemone apennina*, &c., or in the gynaecium of *Ficaria verna*, *Anemone apennina*, or *Clematis Vitalba*, is the periodic character of the variation curves with secondary modes grouped around the primary and almost always corresponding to numbers which are multiples of three (cf. Salisbury, 'Ann. Bot.', vol. xxxiii, pp. 47-79, 1919, and vol. xxxiv, pp. 107-16, 1920).

TABLE I.

*Gynaecium of Alisma plantago.*

Number of Carpels.	Plant A.	Plant B.	Plant C.	Plant D.	Plant E.	Plant F.	
9	1						1
10	0						—
11	0						—
12	1						1
13	0						1
14	2		—	—	—	1	1
15	4	—	1	—	6	2	4
16	3	3	1	1	3	6	17
17	18	3	2	1	3	16	26
18	55	2	48	13	15	47	86
19	44	8	64	21	67	80	265
20	35	20	53	35	44	33	214
21	25	47	25	26	26	12	181
22	8	47	25	56	20	3	176
23	4	42	5	34	10	—	99
24	0	30	1	23	4	—	62
25	—	28	—	13	2	—	43
26	—	12	—	2	2	—	16
27	—	4	—	1	1	—	6
	—	2	—	—	—	—	2
Total							1,200

No one disputes the essentially trimerous character of the Monocotyledonous flower, and we here find it exhibiting a type of variation curve similar in character to that met with in the Ranunculaceous plants above mentioned.

It will be noted that the actual range observed for the number of carpels was from nine to twenty-seven, the lower limit thus being a multiple of three. This, it may be recalled, was also the case for a similar number of fruits of *Clematis Vitalba* and for the stamens of *Eranthis* and *Ficaria* (Salisbury, loc. cit.). For the individual plants the range varies considerably, and on calculating the standard deviation for plants A and D they are found to be 1.85 and 1.69 respectively.

The solitary example of staminal variation mentioned above was a flower with seven stamens, the supernumerary stamen being situated

between, and to the outside of, one of the normal antisepalous pairs of stamens, thus recalling the condition recorded by Buchenau for *Butomus umbellatus* (cf. Eichler, 'Bluthendiagramme I', p. 101). The question as to whether or no these supernumerary stamens represent the outer of two suppressed whorls will be deferred till we have considered the staminal variation in *Sagittaria* and *Butomus*.

(B) ECHINORDORUS RANUNCULOIDES, L.

The material for the study of this species was chiefly obtained from Wisley and from the dune slacks at Southport. Apart from these wild sources, however, a considerable number of plants were raised from seed under as natural conditions as possible. The plants from Southport were a dwarf form two or three inches high only, and have, therefore, been treated separately. Those from Wisley were well-grown plants of the normal type from six to eighteen inches in height.

The flowers of this species differ from those of *A. plantago* in the fact that the six stamens are situated in pairs opposite the petals, and with respect to the carpels, which are not borne in one plane, but in a spiral manner on the elongated receptacle.

The lowest number of carpels observed was a multiple of three, namely twelve. The highest number was eighty-four, but the flower in question was almost certainly fasciated, so that the normal range is probably between twelve and forty-four.

These data show clearly that though there is, as one would expect, a marked tendency for the number of carpels to be some multiple of three, yet departures therefrom are almost as frequent as in the meristic variation of the stamens or carpels of Ranunculaceae.

The departures from the trimerous condition represent 48.5 per cent. of the total number of examples. Comparing this figure with those for gynaecea of *Clematis Vitalba* and *Ficaria verna*, we find that for these the percentage frequency of carpels not corresponding to a multiple of three is 62 per cent. in the case of *C. Vitalba*, and 60 per cent. in the case of *F. verna*.

The departures from the trimerous condition in the Ranunculaceous plants was attributed to fission, fusion, and abortion. That this last plays an important part in bringing about similar departures in *Echinodorus ranunculoides* is indicated by the high proportion of partially aborted carpels. These were met with in no less than 22 per cent. of the flowers examined, and the number of such abortive carpels in a single flower varied between one and sixteen. Here, as in *Clematis Vitalba*, there is a tendency for the number of abortive carpels to increase with increase in the total number of carpels.

TABLE II.

*Meristic Variation in the Gynaecium of Echinodorus ranunculoides.*

<i>Number of Carpels.</i>	<i>Number of Flowers. Wisley (Normal Form).</i>	<i>Number of Flowers. Southport (Dwarf Form).</i>	<i>Totals.</i>
12	0	2	2
13	0	4	4
14	0	1	1
15	0	2	2
16	0	5	5
17	0	7	7
18	0	18	18
19	0	13	13
20	0	10	10
21	3	13	16
22	0	17	17
23	1	15	16
24	6	23	29
25	0	8	8
26	1	12	13
27	14	10	24
28	9	5	14
29	11	5	16
30	42	13	55
31	17	2	19
32	9	1	10
33	17	2	19
34	8	5	13
35	5	0	5
36	26	4	30
37	5	1	6
38	9	2	11
39	8	0	8
40	1	0	1
41	3	0	3
42	3	0	3
43	1	0	1
44	1	0	1
Totals	200	200	400

As might be expected, the range for the depauperate Southport form exhibits a lower minimum and a lower maximum than for the normal type. The standard deviation for the latter is 4.4, and the arithmetic mean 32. For the depauperate type the arithmetic mean is 22, and the standard deviation 5.5. Comparison of these data with those for *Alisma plantago* shows a much higher value for  $\sigma$  in the case of *E. ranunculoides*. The arithmetic means for the number of carpels in the dwarf form of *E. ranunculoides* is very close (22) to that for plant D of *Alisma plantago* (21), yet the standard deviation of the latter is barely one-third of the standard deviation of the former. In both instances we are dealing with the same number of examples, so that the difference in dispersion is in all probability a real one.

The 'curves' shown in Fig. 3, representing the variation in the Wisley flowers as a whole, and also in those from a single plant, bring out the strikingly periodic character both in the 'population' and in the individual. Comparison of the main 'curve' with that for the androecium of *Eranthis hyemalis*, which has a similar numerical range and a primary mode also at 30 (cf. 'Ann. Bot.', vol. xxxiii, p. 55, Fig. 7), reveals the essentially similar variation in the two cases.

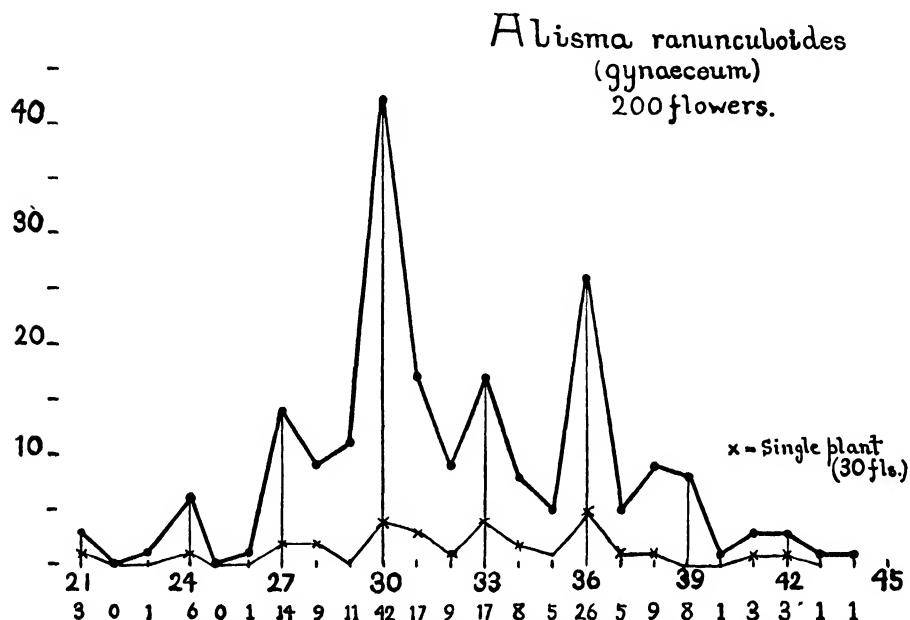


FIG. 3. Meristic variation in the gynaecium of 200 flowers, from the Wisley locality, of *Echinodorus* (*Alisma*) *ranunculoides*. Note the periodic character of the 'curve', both for the 'population' and the individual plant. Ordinates represent the number of examples, and abscissae the number of carpels.

### (C) *SAGITTARIA SAGITTIFOLIA*, L.

The inflorescence of this species commonly exhibits from three to five whorls, of three flowers each, which alternate at successive nodes. More rarely as many as five flowers may be present in a whorl. The flowers are functionally unisexual. The lowest whorl consists of female flowers and the second whorl may also be entirely female or consist of both male and female flowers, whilst the upper whorls are functionally male.

The conditions that determine the sex of the flower are possibly nutritional in the main, but in any case it would seem that the female function is determined by some factor or factors which decrease in intensity from the base of the inflorescence upwards. This can best be illustrated by describing the organization of one of the mixed inflorescences which were examined in detail. First, however, we may note the invariable presence

of abortive carpels in the male flowers and the occasional presence of non-functional stamens in the female flowers: facts which are sufficient evidence of the derivation of the functionally unisexual flower from an hermaphrodite prototype. The inflorescence in question exhibited five whorls of flowers of three members each, but the flowers of the ultimate whorl were too small to be dissected with accuracy. The organization of the flowers from below upwards is shown in the following table:

TABLE III.

	Basal whorl (Carpels 660)	(Carpels 500)	(Carpels 500)
All female flowers			
Second whorl			
female flower (carpels 350)	male flower (stamens 30, carpels 30)	male flower (stamens 30, carpels 13)	
Third whorl			
male flower (stamens 29, carpels 11)	male flower (stamens 29, carpels 8)	male flower (stamens 27, carpels 8)	
Fourth whorl			
male flower (stamens 27, carpels 6)	male flower (stamens 27, carpels 6)	male flower (stamens 25, carpels 6)	
Fifth whorl			
male flower (stamens 24, carpels ?)		(†)	(†)

From this it will be seen that there is a progressive diminution in the number of carpels from below upwards, the lowest flower having 660 fertile carpels, whilst in the penultimate whorl of male flowers the number of abortive carpels is only six. There is a similar diminution in the number of stamens, but this is not nearly so marked as in the gynaeceum. Such numerical gradation is probably a general phenomenon which has, for example, been recently studied with respect to the number of florets in the lateral capitula of the Compositae, by Szymkiewicz ('Acta Soc. Bot. Poloniae', vol. I, No. 3, 1923).

*The perianth.* Variation in the perianth of *Sagittaria* is decidedly rare, but two departures from the normal were encountered in which the typical perianth of two alternating whorls of three members each was modified as a consequence of partial or complete fission of one of the perianth segments. The first of these was a female flower in which the odd sepal, situated on the side remote from the inflorescence axis was replaced by two sepals. In the second instance the calyx was normal, but the adaxial petal was divided almost to its base. Those instances where more than three flowers are present in a whorl would appear to be due to a similar bifurcation of one or more of the bracts and the buds in their axils. It will be recalled that fission of the perianth members has been shown to be of frequent occurrence in various members of the Ranunculaceae (cf. Salisbury, loc. cit.).

*The androecium.* The stamens are arranged in whorls, the outermost consisting of six members arranged in pairs opposite the sepals, thus

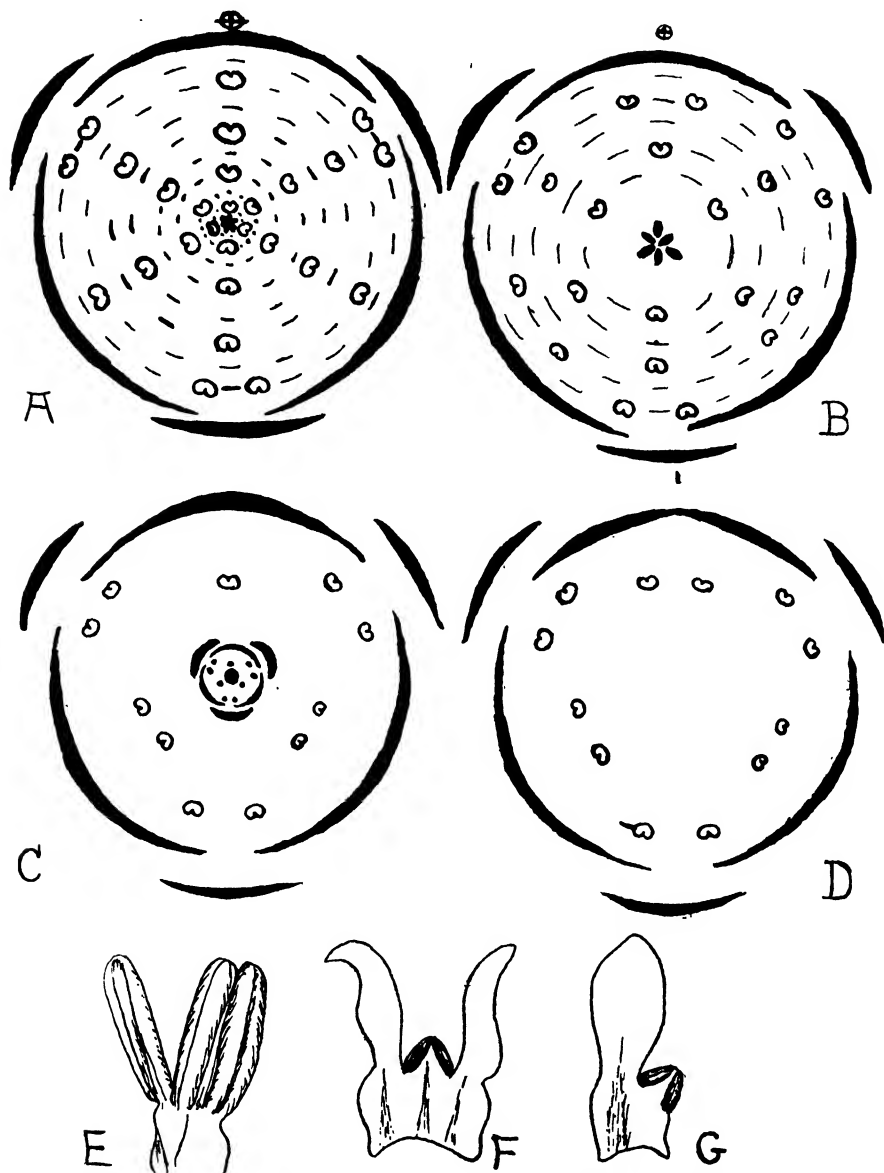


FIG. 4. *Sagittaria sagittifolia*. A. Diagram of normal flower showing eight whorls of stamens and six carpels. B. Diagram of flower with five whorls of stamens, the second whorl exhibiting the same dedoublement as normally exists in the outermost whorl. C. Abnormal proliferated flower with eleven stamens and the ovary replaced by a second flower with a normal perianth, nine stamens, and a flower-bud in its centre. D. Diagram of flower with one member of the inner perianth whorl bilobed, twelve stamens (one petaloid). E. Branched stamen with three anthers. F and G. Petaloid stamens.

corresponding to the six stamens normally present in *Alisma*. These six stamens are followed by a varying number of whorls, each of three stamens, which exhibit regular alternation. If, therefore, we accept the usual interpretation of the outer whorl as representing a trimerous whorl, of which each member has undergone congenital fission, then the normal flower

*Sagittaria*  
*sagittifolia*  
94 ♂ fls.

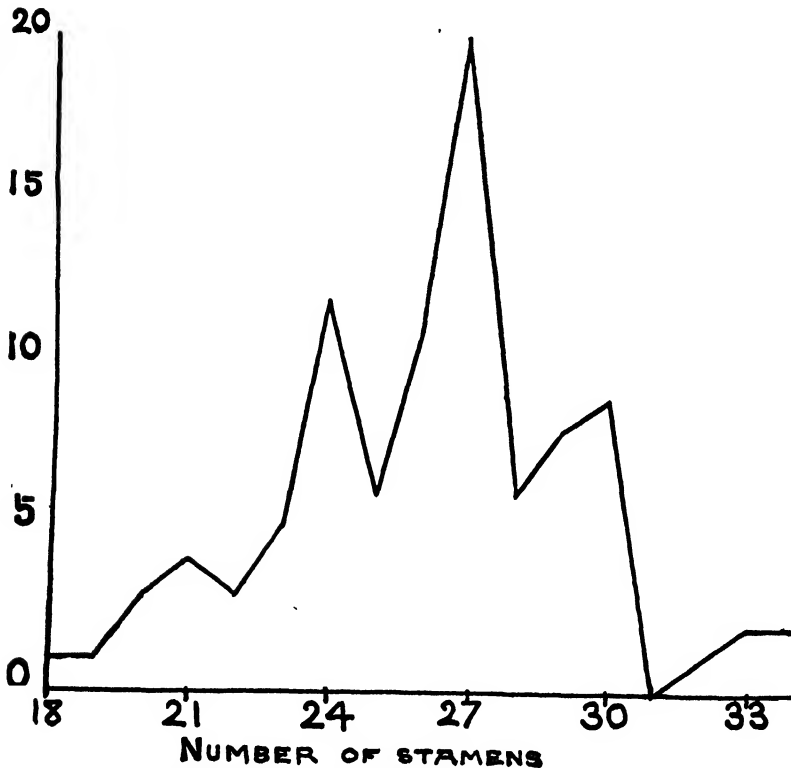


FIG. 5. *Sagittaria sagittifolia*. Meristic variation of ninety-four male flowers, showing primary mode at 27 and secondary modes at 21, 24, and 30.

of *Sagittaria* is trimerous throughout with regular alternation such as we commonly find in Monocotyledonous flowers. Examination of a considerable number of flowers, whilst confirming this view, also shows that departures are by no means uncommon. In one flower, for instance, the second whorl exhibited the same congenital fission as the first, the three pairs of stamens of the second whorl alternating with the antisepalous pairs of the first whorl. The subsequent whorls, to the number of three, were quite normal (Fig. 4, B).



One plant of *S. sagittifolia* was found in which the flowers all exhibited proliferation, the gynaeceum being replaced by a flower bud. These flowers showed a number of features of interest. Firstly, it may be noted that the flowers exhibited perfect alternation throughout, the sepals of the inner flower alternating with the innermost stamens of the outer flower (Fig. 4, C and D). The condition presented is, in fact, exactly comparable to that so commonly met with in the double form of *Arabis albida*. Secondly, in all these flowers the number of stamens was unusually small,

TABLE IV.

*Meristic Variation in the Androecium of Sagittaria sagittifolia, L.*

<i>Number of Stamens.</i>	<i>Number of Examples.</i>	
9	2	} abnormal flowers
10	2	
11	1	
12	1	
18	1	} Normal male flowers
19	1	
20	3	
21	4	
22	3	
23	5	
24	12	
25	6	
26	11	
27	20	
28	6	
29	8	
30	9	
31	0	
32	1	
33	2	
34	2	
Total 100		

and it is significant that the minimum number was nine, and the arrangement identical with that normally found in *Butomus umbellatus* or *Echinodorus parvulus*. In another example, one of the three pairs of stamens of the outermost whorl was replaced by a single stamen, thus affording striking support for the current interpretation. A third noteworthy feature of these abnormal flowers was the frequent occurrence of petaloid stamens. These usually bore the anther to one side of the petaloid structure (Fig. 4, G), but in one instance (Fig. 4, F) there were two petaloid enlargements symmetrically placed one on either side of the connective bearing a terminal anther. Such stipule-like appendages arising from the sporophyll base recall the similar condition normally present in other Monocotyledonous flowers, such as *Allium cepa*, *Cali-*

*phruria* (Amaryllidaceae), *Barbacenia* (Velloziaceae), &c. Similar appendages also characterize some Dicotyledonous genera as, for example, *Crambe*, *Deutzia*, and *Hermannia* (Sterculiaceae), and may indicate the survival of a primitive potentiality in the Angiosperms as a whole.

The meristic variation in the androecium of *Sagittaria* was studied in ninety-four examples of normal male flowers and in six abnormal proliferating ones, the data being presented in Table IV and Fig. 5.

The variation is thus of a periodic character with a normal range of from 18 to 34, a primary mode at 27, and secondary modes at 24 and 30. Departures from the trimerous condition may be attributed to fission and fusion. The following examples of flowers with branched stamens sufficiently illustrate the frequency of modifications due to the same causes as are so evident in Ranunculaceous flowers.

P 3-3	A 26	(one branched stamen)	= 27	G ?
	28	" "	= 29	G ?
	29	" "	= 30	G 8
	30	" "	= 31	G 9

In one flower three anthers were present on one filament (Fig. 4, E), showing the possibility of trifurcation in place of bifurcation (cf. *Echinodorus humilis*, where three stamens occur opposite to each petal). The number of abortive carpels in the male flowers was determined in twenty examples, and was found to range from 3 to 30. It will be seen from Table V that even this small number is sufficient to show that there is a direct correlation between the number of carpels and the number of stamens, both tending to augment or diminish together.

TABLE V.

*Relation between Number of Stamens and Number of Carpels in Male Flowers of Sagittaria sagittifolia.*

<i>Number of Stamens.</i>	<i>Number of Carpels.</i>	<i>Number of Examples.</i>
19	3	1
20	10	1
24	3	1
24	12	1
25	6	1
26	7	2
26	9	1
27	6	2
27	8	1
27	9	1
27	10	1
28	6	1
29	8	1
29	11	1
30*	8	1
30	13	1
30	30	1
31	9	1

The total number of parts in any one male flower varied between 28 and 66, the most frequent number being 39 (P 3 + 3, A 27, G 6). In 45 per cent. the total was some multiple of three. This, taken in conjunction with the perfect trimery of the proliferating flowers, suggests that the apical meristem, though multicellular, functions in a similar way to a three-sided apical cell.

In the male flowers, where the number of abortive carpels is few, the whorled character of the floral parts is perfectly clear. Nevertheless one fully recognizes that the members of any one whorl are actually produced in succession. The whorled arrangement is, in fact, dependent on a certain rhythm in growth of the thalamus. Clearly the chief elongation of this latter must take place between the production of successive groups of three lateral members. When, however, the number of carpels is large they are found to be arranged in a spiral manner. It is not reasonable to suppose that the mode of formation of the lateral organs varies with the number of these produced, yet we find that the phyllotaxis may become very high when the number of carpels is large. The only possible conclusion seems to be that the spiral arrangement and its phyllotaxis is dependent on purely mechanical conditions determined by the interaction of (1) the rate of production of the lateral organs, (2) the rate of elongation of the thalamus. When the former is high and the latter is low a high phyllotaxy results. Hence in a definitely limited organ like a flower, where the total number of parts produced can be accurately determined, the mode gives us a definite indication of the manner of segmentation at the growing-point, being usually a multiple of the number of parts formed in a single cycle.

#### (D) *SAGITTARIA OBTUSA.*

This North American species differs chiefly from the preceding in the fact that the male and female flowers are borne on separate individuals. The male plants alone appear to be naturalized in Europe, and only a few plants of this sex have been examined. The observed range in the number of stamens was from 30 to 52, and the mode would appear to be 42.

Owing to the small number of examples, no great stress can be laid on these figures, but it is clear that in this dioecious species the number of stamens is much larger than in its congener, the three antisepalous pairs of stamens being here followed by about twelve whorls of three members each, in place of the seven whorls characteristic of *S. sagittifolia*.

A second feature of interest is that in *S. obtusa* the dehiscence of the stamens clearly varies between being definitely extrorse and marginal.

## (E) BUTOMUS UMBELLATUS.

The flowers of this species exhibit little tendency to vary, and hundreds of specimens can be examined without finding any departures from the mode in which three pairs of antisepalous stamens are followed by a single whorl of three antipetalous members, and these in turn by two trimerous whorls of carpels.

The outer perianth whorl sometimes exhibits a supernumerary member,

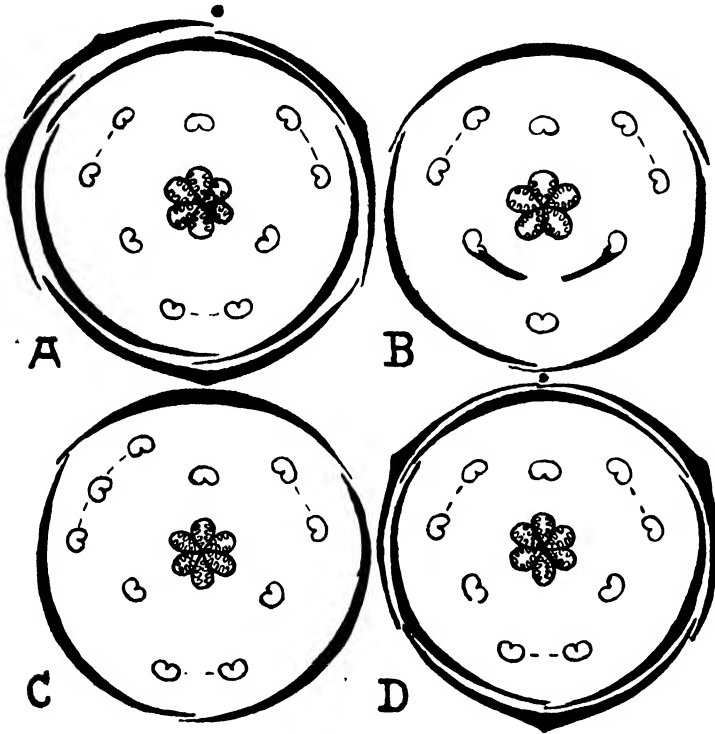


FIG. 6. *Butomus umbellatus*. A. Floral diagram of flower with supernumerary segment in outer perianth whorl. B. Diagram (outer perianth omitted) of flower with a single stamen replacing one of the antisepalous pairs and two of the inner stamens petaloid. C. Diagram (outer perianth omitted) of flower with three stamens in place of one of the antisepalous pairs. D. Diagram of normal flower.

which is clearly derived by fission, as shown in the flower, of which the floral diagram is given in Fig. 6, A.

The usual floral structure is shown in Fig. 6, D. The three pairs of antisepalous stamens arise simultaneously, as Payer observed, and doubtless represent the congenital dedoublement of a trimerous whorl.

In Fig. 6, C, is shown the diagram of a flower (the calyx here and in B has been omitted) with ten stamens, the additional member being situated between the members of an antisepalous pair. It seems reasonable to

regard this as representing a congenital fission into three instead of two members. In this instance there did not appear to be any difference in level of origin for the three members, but Buchenau found 10- and 11-androus flowers in which the one or two extra stamens were inserted opposite the sepals, but at a lower level than the antisepalous pair. We have already noted (*p.*) the same condition in *Alisma plantago*, and therefore we may suppose that whatever explanation is adopted should apply to both.

Eichler regarded this as a displacement outwards of the middle member of the product of a fission into three, a view which our specimen and the occurrence of trifurcated stamens support. Moreover, in *Echinodorus humilis* the three antisepalous groups would seem to indicate a normal trifurcation. Buchenau, however, supposed that these represented partial reversion to an ancestral condition with an additional whorl, and Čelakovský, to overcome the difficulty of the lack of alternation that such a supposition involved, hypothecated two additional external whorls, of which these supernumerary stamens represented the outer. In the allied family of the Hydrocharitaceae we find two trimerous whorls of staminodal groups outside the whorl of paired stamens.

It seems hardly probable, in view of the extraordinary uniformity of androecial construction in most of the Helobiales, that the six antisepalous stamens are not homologous in the various members. We must, then, suppose, if we accept Čelakovský's view, that two outer whorls are suppressed in every case. Comparative study of the various members of the Cohort, however, shows that there has in general been suppression from within outwards, and not in the reverse direction. Even in the same species the number of whorls of stamens may vary considerably, but, if we except the flowers of *Echinodorus ranunculoides* and *E. humilis* and the supernumerary stamens of *Butomus* and *Alisma*, the variations in no case give any indication of additional whorls to the outside. The sole criterion for such an assumption is the male flower of *Stratiotes*, which raises more difficulties than it would solve. On the other hand, Eichler's interpretation merely assumes a displacement such as we know frequently takes place, and a fission that, as we have seen, is evidently a group tendency.

The view that the antisepalous pairs of stamens are the result of congenital fission receives support from the specimen illustrated in Fig. 6, B, where one of these pairs is replaced by a single stamen. A further feature of this flower was that two of the stamens were partially petaloid, and in another flower three petaloid stamens were present.

The total range observed in the number of stamens was from 8–11, and in the number of carpels from 4–6.

*Butomus* is peculiar in the possession of ovules scattered all over the surface of the carpel except for the region of the midrib, a rare

condition recalling *Nymphaea* amongst the Ranales. The number of ovules is large, though frequently a high proportion are abortive. The range observed was from 36 to 119 per carpel, with a mean of  $88 \pm 3.5$ . As there are quite commonly thirty flowers in an inflorescence, and two or three of these will be produced on a plant, about 8,000 seeds may be produced in a season. In eleven of the carpels examined, however, the number of abortive ovules was very high. In two instances all the ovules were infertile, and in the other nine the number of fertile ovules did not exceed thirteen. In this connexion we may note that *Butomus*, like the majority of aquatics, propagates very largely by vegetative means.

#### (F) STRATIOTES ALOIDES, L.

Only female flowers of this species have been examined, and for this material I am indebted to Miss Barbara Russell-Wells, Ph.D.

The chief feature of the flowers of both sexes, as pointed out by Eichler, is the presence of two whorls of staminodes of three members, each situated immediately within the perianth. In the female flower these staminodes may occasionally bear anthers, so that their morphological character does not seem to admit of question. Eichler ('Bluthendiagramme I', Fig. 43, B), following Rohrbach, figures the staminodes as forming two alternating whorls, of which the outer is antipetalous. In the material examined, far from being able to confirm this, the reverse arrangement appeared to obtain, and this was supported by examination of transverse sections. It is, of course, not impossible that both conditions may occur, though this hardly seems likely, and the course of the vascular bundles strongly supports the view that alternation is complete throughout.

A point to which we would call attention here is the extreme variability in the number and grouping of the filiform segments into which the staminodes are divided (cf. Fig. 7, A and C). The flower represented in Fig. 7, A, shows the usual six groups, but whilst in four instances these each consist of four segments the other two groups consist of seven each. Actually, as shown below, the total number of filiform segments, in only nineteen examples, ranged from 18 to 30, and the individual groups consisted of from one to seven branches. The six groups of four filaments each shown by Eichler, giving a total of twenty-four, is quite probably normal, though actually the most frequent number in these specimens is twenty-two. This and the occurrence of one example with a total of thirty (Fig. 7, A) suggest the possibility that larger numbers of specimens might reveal secondary modes. It would appear, then, that we have here further evidence for the tendency towards fission, exhibited in varying degrees, and thus affording additional support to the view that supernumerary stamens are derived in this manner. In this connexion we may note that

in the male flower, according to Eichler, the fertile stamens belonging to the inner whorls may undergo fission, whilst in the female flowers of *Hydrocharis* the staminodes may be single or double.

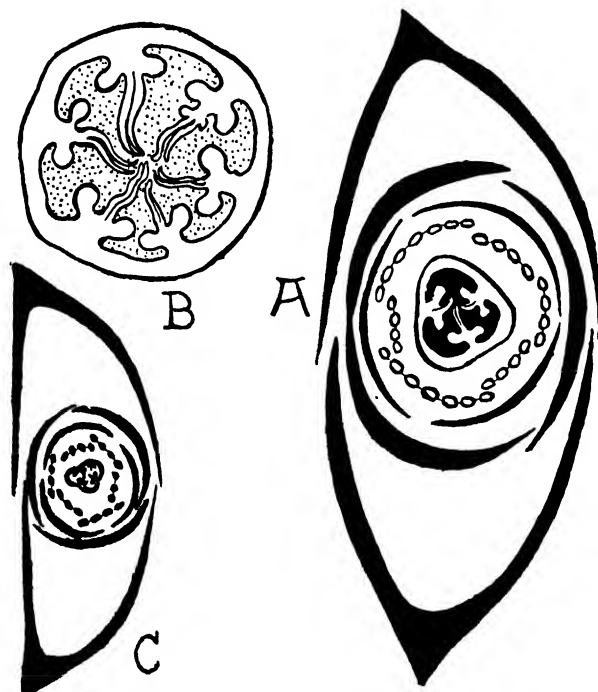


FIG. 7. *Stratiotes aloides*. A. Diagram of female flower with two of the outer perianth segments fused and three carpels. B. Ovary of flower with six carpels. C. Diagram of flower with four carpels.

TABLE VI.

*Variation in Carpel and Staminode Number in 19 Specimens of Stratiotes.*

Number of Staminodes.	Number of Carpels.				Total.
	3	4	5	6	
18			1		1
19					
20		1			1
21			1	2	3
22		1		5	6
23			1	1	2
24				2	2
25				2	2
26				1	1
27					
28					
29					
30	1				1
	1	2	3	13	19

The normal condition of the ovary exhibits six carpels (Fig. 7, B), but five, four (Fig. 7, C), or even three (Fig. 7, A) may occur, and one instance of a partially-developed carpel was noted.

The incidence of fusion is shown by the flower represented in Fig. 7, A, in which two of the calyx segments are replaced by a bilobed sepal.

Here too, then, it is apparent that there are frequent departures from the trimerous condition, and that these may be attributed to fission, as seen in the staminodes, to suppression, as seen in the carpels, or to fusion, as shown by the calyx.

Considering the Helobiales as a whole the *androecium* is manifestly derived from a succession of alternating whorls of three members each, and there seems little reason to doubt that reduction in the number of these whorls has been one of the chief features of the group. Thus we could construct a series of male flowers from *Limnobium* with sometimes as many as five whorls, the innermost staminodal, through *Hydrocharis* (3 + 3 + 3 + 3-std.), *Elodea* (3 + 3 + 3-std.), and *Lagerosiphon* (3 + 3-std.), to *Triglochin montevidense* or *Wiesneria*, with only three stamens in the antisepalous positions (cf. Eichler, loc. cit., Buchenau, 'Pflanzenreich'). Moreover, the staminodal condition of the innermost whorl in *Limnobium*, *Hydromystria*, *Hydrocharis*, *Elodea*, *Lagerosiphon*, &c., and the monothecous condition of the penultimate whorl of *Hydrocharis* clearly point to the centrifugal character of this reduction tendency, which proceeds even a step farther in *Vallisneria*, where only two of the three stamens are fertile.

But there is another tendency also exhibited by the group, namely, towards staminal fission normally seen in certain members as a congenital dedoublement of the outermost whorl of stamens. In *Sagittaria* this whorl of six stamens may be followed by as many as twelve whorls of three members each, whilst in *Alisma* the antisepalous whorl of six stamens stands alone. Intermediate conditions are presented by *Echinodorus* and *Butomus*, and here, too, it seems reasonable to suppose that reduction in the centrifugal direction has taken place.

We have seen, too, that not only can the whorl of six antisepalous stamens be replaced by a whorl of three single stamens, but that the reverse condition with respect to the second whorl may occur. In *Stratiotes* the two staminodal whorls exhibit this fission in a high degree, and it is the third whorl which exhibits the congenital fission. Moreover, it is significant that it is amongst these types with normal fission of the outermost whorl that abnormal fission is most commonly met with. It is not improbable that the numerous stamens of *Limnocharis* represent an extreme development of this fission tendency.

It is these two opposing tendencies, together with fusion of parts, that combine to produce the irregularities which, even in the stereotyped



Monocotyledonous flower, result in the marked departures from the trimorous condition. The variation curve produced is not, however, unimodal, but, in a more or less marked degree, is of a periodic character, and presents so striking a resemblance to the periodic curves of Ranunculaceous flowers as to constitute a cogent argument in support of the interpretation of the Ranunculaceae as exhibiting a hidden trimery.

#### COMPARISON OF THE HELOBIALES AND THE RANALES.

In view of the facts here adduced it seems desirable to summarize, in the light of present knowledge, the resemblances between the Helobiales and the Ranales in general, and in particular between the Ranunculaceae and the Alismataceae.

Before doing this, however, we may emphasize the importance of rare or even abnormal features as indications of relationship. No feature is without significance as an indication of the potentialities of the group or the individual in which it occurs. Even pathological conditions, representing as they do the resultant of the interaction of the physiological complex of the organism and an abnormal environment, whether internal or external, may have a value as the visible clue to a common physiological inheritance. Exceptional features in two different groups, however unique, do not of course necessarily indicate any relationship, yet if present in conjunction with more normal and widespread features they do add materially to the weight of evidence.

It is with this principle in mind that we would emphasize the presence in both the Butomaceae and the Nymphaeaceae of the unique type of placentation with ovules scattered over almost the entire inner surface of the carpel, and the presence of latex in the Nymphaeaceae and Alismataceae, in which connexion we may recall the very Monocotyledonous type of flower in *Cabomba*. Or again we may note the occurrence of the Monocotyledonous type of embryo in *Ficaria verna*. Such features have, in our opinion, the greater significance when considered in relation to the more general resemblances with which we shall now deal.

First of all considering the vegetative and anatomical characters, we now recognize that the Monocotyledons as a whole are not distinguished by the total absence of a fascicular cambium, but rather by its vestigial character. Such a vestigial cambium has now been found in a large number of members of the group belonging to all the Monocotyledonous cohorts with the exception of the saprophytic Triuridales. In the Helobiales a vestigial cambium is known from the Juncaginaceae, where, as S. Andersson (cf. 'Bot. Centrbl.', xxxviii, pp. 586 and 618, 1889) and T. G. Hill ('Ann. Bot.', xiv, pp. 83-107, 1900) have shown, the cambium is well developed. Recently Mrs. Arber has recorded a cambium also from the Alismataceae (*Sagittaria*

*sagittifolia*), Hydrocharitaceae (*Hydrocharis morsus-ranae* and *Stratiotes aloides*), Aponogetonaceae (*Aponogeton distachyum*), and Potamogetonaceae (*Potamogeton natans* and *P. lucens*) (cf. Arber, 'Ann. Bot.', xxxvi, pp. 251-6, 1922; also *ibid.*, xxxi, pp. 41-5, 1917; xxxii, pp. 87-9, 1918; xxxiii, pp. 459-65, 1919). This is the more striking in view of the reduced character of the bundles in this cohort associated with the prevalent aquatic habit.

The flowering axes of *Triglochin*, *Potamogeton*, *Damasonium*, &c., present a strikingly Dicotyledonous type of organization with a single ring of bundles, whilst the flowering axes of several members of the Ranunculaceae (e.g. *Anemone japonica*, *Thalictrum* spp., *inter alia*) exhibit a Monocotyledonous type of organization with the bundles arranged in more than one ring: a feature also encountered in the Berberidaceae, Calycanthaceae, and Nymphaeaceae. Moreover, it is in the Ranunculaceae that we find the nearest approach to the Monocotyledonous bundle with a cambium that may be almost vestigial and the phloem tending to be flanked by metaxylem elements.

In the Helobiales the leaf arrangement may be distichous as in Potamogetonaceae, a  $1/3$  arrangement as in *Butomus*, or  $2/5$  as in the vegetative region of *Alisma plantago*. Amongst the Ranales  $2/5$  and higher types of phyllotaxy are the rule; but the  $1/3$  arrangement occurs (e.g. *Eranthis*), and the distichous phyllotaxy is characteristic of many Anonaceae and is seen in our native *Ranunculus flammula*. Even in *Clematis*, seedlings sometimes exhibit whorls of three leaves at each node. It is characteristic of Monocotyledons that the first leaf of a secondary axis is adjacent to the primary axis, whereas in most Dicotyledons the first leaf of the secondary axis is lateral with respect to the primary axis. In Anonaceae and Nymphaeaceae, however (as also in the Aristolochiaceae), the orientation characteristic of Monocotyledons obtains (cf. R. E. Fries, 'Ein unbeachtet gebliebenes Monokotyledonenmerkmal bei einigen Polycarpicae', 'Ber. d. Deut. Bot. Ges.' 29, pp. 292-301, 1911).

Turning to the floral organization, we have seen that the flowers of the Helobiales with numerous parts are trimerous in their construction but exhibit a meristic variation 'curve' that may be periodic in character and almost identical in general form to those of some members of the Ranunculaceae. Amongst the Monocotyledonous families one can recognize that the dimerous condition is the normal derivative of the trimerous. This is well seen in the gynaeceum of *Carex*, in the flower of the Gramineae and amongst the Helobiales themselves in the Potamogetonaceae or the dimerous flower of *Tetroncium*. In the Ranales the like tendency is to be expected and is shown by members of the Anonaceae and Berberidaceae (*Epimedium*). It is instructive in this connexion to note the entire absence of periodicity in the meristic variation of the floral whorls of *Paris quadrifolia* and the comparative rarity of the trimerous condition (cf. Fig. 8), which latter is

the more striking from the close affinity of this genus to the genus *Trillium*, where, though tetramerous flowers occur abnormally, trimery is the rule (cf. R. R. Gates, 'A Systematic Study of the genus *Trillium*, its variability, and

	3	4	5	6	7	8	9	10	11	12	
Inv	1	1211	259	29							
O.P.	8	1464	23	0							
I.P.	24	1470	6	0							
A.	—	—	—	1	12	1402	71	13	1	0	
G	11	1465	23	1							

PARIS  
QUADRIFOLIA  
(J.S.HENSLAW 1832)  
1500 fls.

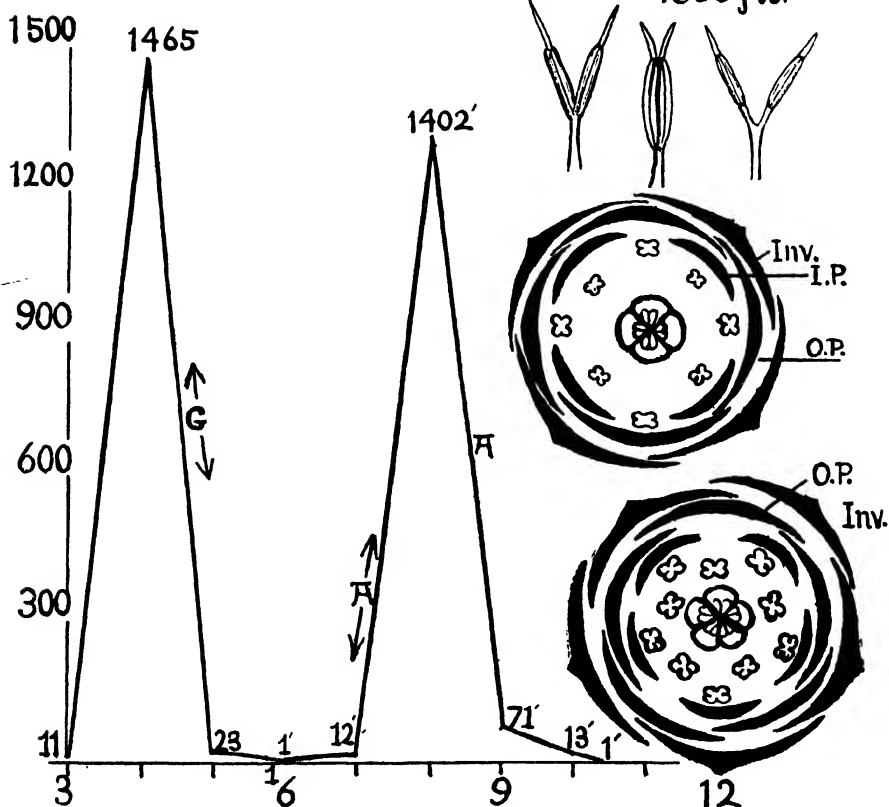


FIG. 8. *Paris quadrifolia*. Variation curve based on the data of J. S. Henslow for 1,500 flowers. On the right are shown three abnormal stamens and diagrams of a tetramerous and a pentamerous flower.

its relation to *Paris* and *Medeola*', 'Ann. Missouri Bot. Gard.', iv, pp. 43-92, 1917). The case of *Paris* shows to what extent the derivation from an ancestral trimerous condition may become obscured.

The Helobiales which in so many other respects show features we commonly associate with Dicotyledons are almost unique amongst Monocotyledons in the differentiation of the perianth into an outer protective and an

inner attractive whorl. The recorded abnormalities of Monocotyledonous flowers in which both the perianth whorls exhibited staminody suggest that the perianth, in some families at all events (e. g. Liliaceae, Iridaceae, Orchidaceae), is homogeneous in origin. In the Helobiales it is possible that the differentiation is due to a heterogeneous origin, but there are, so far as the writer is aware, no 'abnormalities' which shed any light on the origin of the two whorls. In either case we are justified in drawing a comparison between the almost certainly staminodal petals of the female flower of *Hydrocharis*, each of which bears a nectary protected by a scale, and the similar nectary-bearing petals of *Ranunculus*, where the staminal origin can scarcely be questioned. As the writer has shown ('Ann. Bot.', xxxiii, pp. 74, et seq.), the perianth of the Ranunculaceae may be either heterogeneous or homogeneous in origin. So far there is no evidence that in this family the homogeneous perianth is in any case derived by modification of stamens, as it would appear to be in some at least, if not most, of the Monocotyledons, and such a difference in the origin of the homogeneous perianth may indeed be an important difference between the majority of Monocotyledons and Dicotyledons. If, however, evidence should be forthcoming of the origin of the outer perianth whorl in the heterochlamydeous Helobiales from foliar structures the approach to the Ranales would be considerably emphasized. If, however, we accept the view of de Candolle recently elaborated by Mrs. Arber (cf. 'Ann. Bot.', xxxii, pp. 465-501) regarding the phyllodic nature of the Monocotyledonous 'leaf', this not only constitutes one of the most important distinctions between the two groups, but renders it less likely that recognizable reversions to the foliar prototype should occur.

The potentiality for the development of petals from stamens which has become fixed in so many Ranunculaceae is presented as an abnormality in *Sagittaria*, *Butomus*, &c., as in other Monocotyledonous and Dicotyledonous families. The unisexual condition derived by partial or complete abortion of the male or female organs, which is frequent in the Helobiales, is found in the Ranunculaceae in species of *Clematis* (*C. Colensoi* and other New Zealand species) and in *Xanthorrhiza*. In this connexion we may note that Velenovsky has recorded the occurrence of plants of *Ranunculus acris* with flowers in which the stamens were aborted or absent ('Eine interessante Missbildung in den Blüten des *Ranunculus acris*', Oester. Bot. Zeitschr., pp. 244-5, 1900).

The unusual feature of numerous stamens characteristic of Ranunculaceae is a feature also of some Alismataceae and Butomaceae (*Echinodorus longistylis*, *Limnocharis*, *Hydrocleis*), and in both families we note the same tendency for the cyclic condition to give place to the acyclic under the mechanical conditions imposed by a multiplicity of parts.

Branching of the stamens we have recognized as of frequent occurrence in both groups, whilst staminody of the external members is met with in the

male flowers of *Stratiotes* and *Pulsatilla*, of the internal in several of the *Helobiales* and in *Aquilegia* in the Ranunculaceae. These features, though of no great importance in themselves, illustrate the common tendencies in the two groups.

The dedoublement of the outer staminal whorl so characteristic of the *Helobiales* has its counterpart in the dedoublement of the whorl of nectaries in *Eranthis* (cf. Salisbury, loc. cit.). Both in the Ranunculaceae and in the *Helobiales* we find the rare conditions of both extrorse and lateral dehiscence of the stamens.

In the gynaeceum both these groups present the rare apocarpous type with numerous free carpels ; but, further, in both there is the same tendency towards fusion as seen in the basal attachment of the carpels in *Damasonium* on the one hand and in *Helleborus* on the other. In this respect the two genera *Nigella* and *Triglochin* afford a striking parallel. In the former we have species such as *Nigella arvensis* fused only in the lower half, *N. gallica* with the greater part of the carpels fused, and *N. damascena* with only the styles free. In *Triglochin* such species as *T. procera* (carpel free), *T. mucronata*, *T. maritimum*, &c., constitute a similar series.

Again, the Ranunculaceae and *Helobiales* agree in containing a sequence of carpel types from the follicle to the achene. Just as in the former group we have the folliculate condition presented by *Helleborus*, *Eranthis*, &c., the achene with a single ovule in *Ranunculus*, *Hepatica*, &c., and the intermediate condition presented in *Anemone* and *Clematis* spp., where the one fertile ovule is accompanied by others which abort : so too in the Alismataceae we find achenes in *Alisma*, follicles in *Damasonium polyspermum*, and a normally bi-ovulate but sometimes uni-ovulate condition in *Damasonium stellatum*.

Agardh drew attention to the fact that the apotropous or epitropous character of the ovule is usually a taxonomic feature of great constancy ('Theoria systematis plantarum', 1858), but both conditions are met with in the Ranunculaceae, and the same variability is shown by the Alismataceae (cf. Warming, 'Sur la valeur systématique de l'ovule', Copenhagen, 1913). A marked feature of the ovules of the Ranunculaceae is the occurrence within one and the same family, sometimes even within the limits of a single genus (e.g. *Delphinium*, *Thalictrum*) of one or two integuments. Thus a single integument encloses the ovule in *Ranunculus*, *Myosurus*, *Anemone*, &c., two are present in *Caltha*, *Aconitum*, *Trollius*, &c., whilst partially fused integuments characterize *Eranthis hyemalis* and *Helleborus foetidus*. The *Helobiales* present the more primitive condition of two integuments with great uniformity, but the allied Triuridales have only one.

Large antipodals are well known as a feature of the Ranunculaceous embryo-sac (in Compositae the large antipodals are clearly connected with their haustorial function). In *Caltha* the antipodals are not only large but

multinucleate, whilst in *Hepatica* as many as twenty-five antipodals may be present (cf. Coulter and Chamberlain, 'Morphology of Angiosperms'). These conditions in the reverse order may well represent a reduction series from a primitive multinucleate condition, and if so would challenge comparison with the numerous antipodals met with in some Monocotyledons.

Nitzschke called attention to the closer resemblance of the embryo-sac of the apocarpous Nymphaeaceae to those of the Helobiales than of any other group, whilst Strasburger has stressed the fact that 'of all the Dicotyledons certain Ranunculaceae resemble most closely the Monocotyledons in the large size of their embryonic cells'.

Coulter and Land have described Dicotyledonous embryos of the Liliaceous genus *Agapanthus* ('Bot. Gaz.', vol. lviii, pp. 509-19, 1914) and have shown that a vestigial second cotyledon may be present in *Sagittaria*.

Finally we may note that *Zostera* and *Ranunculus* agree in presenting the peculiarity of the derivation of the tapetum from potentially sporogenous tissue and that serological experiments support the affinity here suggested (cf. Mez and Gohlke, 'Cohns Beit. Biol. Pfl.', B. 12, p. 172, 1913).

Our comparison might be further extended, but sufficient evidence has been adduced to show that the resemblances between the two groups and the potentialities they exhibit warrant the recognition of a much closer affinity than is indicated by the clumsy, if convenient, two-dimensional system of our current classification. A striking feature of these resemblances is that they comprise parallel variations with respect to features that in other taxonomic affinities are relatively stereotyped. We are therefore led to conclude that both the Helobiales and Ranales have retained a degree of plasticity in organization that is only consistent with the assumption of a common retention of primitive features. Either we must admit a degree of relationship that would warrant a fundamental change in our classification or, if we regard the difference in the nature of the foliar organs and the prevailing but not universal differences in the embryo as justifying the present arrangement, we must assume that the parallelism is a common inheritance from the ancestral Angiosperm stock prior to the origin of its two main divisions.

It is the latter view that seems the more tenable, and which if accepted necessitates the recognition that the primitive Angiospermous flower exhibited a trimerous construction and not a spiral organization as many writers have assumed. The spiral Magnoliaceous flower has often been singled out as the prototype of the pro-angiosperm flower, when in actual fact the evidence derived from the examination of Magnoliaceous flowers points to the primitiveness of the trimerous types in this family and to the spiral types as subsequent derivatives which in some instances at least exhibit an obscured 'trimery'.

The fact that all Monocotyledonous flowers are either trimerous or types derived from the trimerous condition necessitates the assumption of

this type of floral construction in their progenitors. The writer has accumulated evidence from a number of species, in addition to those of which accounts have already been published, showing that the Ranales are essentially a trimerous group and from this condition the pentamerous flower has been derived. Moreover, the trimerous flower is by no means unknown in other of the less specialized Archichlamydeous cohorts and families, as for example in the Polygonaceae and Fagaceae where, too, the normal derivative dimery is encountered. Amongst other families in which trimery occurs sometimes as a marked feature may be mentioned Cephalotaceae, Platanaceae, Cneoraceae, Tremandraceae, Empetraceae, Limnanthaceae, Aristolochiaceae, Passifloraceae, Achariaceae, Datisceae, Begoniaceae, Lythraceae, Combretaceae, and Melastomaceae.

If, then, the Angiospermous stock possessed a trimerous flower retained almost throughout the Monocotyledonous descendants, and here and there even in the main Dicotyledonous line, it follows that this must be due to some basic feature of organization.

The general relation between floral and vegetative phyllotaxy is sufficiently obvious not to need emphasis, but, as so frequently is the case, the reproductive organs depart less from the ancestral condition than the vegetative.

As has already been pointed out in connexion with *Sagittaria* (and similar evidence could be adduced from other groups), the behaviour of the growing-point is most consistent with the conception that the apical meristem of the Angiosperm is a multicellular equivalent of the three-sided apical cell.

The argument for the derivation of the Angiospermous meristem from a three-sided apical cell has been briefly stated by Haberlandt ('Physiological Plant Anatomy', 2nd Eng. ed., note 40, p. 714). The extraordinary prevalence of the single three-sided cell in the lower groups and its occurrence, even if rare, in the embryonic apices of Conifers,<sup>1</sup> and its occasional presence in the roots of Monocotyledons are strong evidence in support of this view. The observations of Bucholz ('Embryo Development and Polyembryony in relation to the Phylogeny of Conifers', 'Amer. Journ. Bot.', vol. vii, pp. 125-45, 1920) make it clear that in some of the more primitive members of the Coniferales the pro-embryo possesses an apical cell (e. g. *Pinus*, *Cedrus*, *Juni-*

<sup>1</sup> Dingler (Über das Scheitelwachstum des Gymnospermenstammes, München, 1882) claimed to have recognized apical cells in the growing-point of adult conifers, and Karschelt, 'Zur Frage über das Scheitelwachstum bei den Phanerogamen' (Pringsh. Jahrb. f. w. Bot., xv, 1884), made a similar claim for Angiosperms. Douliot also concluded that there was a single pyramidal or prismatic apical cell in Gymnosperms (Ann. Sci. Nat., sér. vii, tom. 8, 1890). Groom's observations by means of optical sections of 'cleared' material are adverse to this view (Ber. d. Deut. Bot. Ges., pp. 303-12, 1885), whilst those of Koch (Jahrb. f. w. Bot., xii, 1881) indicate a transitional type of organization. The evidence as a whole is, however, consistent with the derivation of the multicellular meristem from the apical cell.

*perus*, *Tsuga*, &c.), and in *Pinus* at least this has three effective sides. This author states that the 'first apical has only one cutting face, and later, when the embryos have separated, it has three cutting faces, but this apical cell disappears before the embryo has reached 500 cells.

In Angiosperms as in the gametophytes of Mosses the production of numerous lateral organs is commonly accompanied by the development of a high phyllotaxy, but, as pointed out by Henslow ('Trans. Linn. Soc.', vol. vii, p. 134, 1908), in any spiral phyllotaxy except  $1/2$  there are always three leaves in any transverse section: a fact which has considerable significance in this connexion.

If we admit that the meristem of the Angiosperm is the multicellular equivalent of the three-sided apical cell, then the prevalence of the trimerous arrangement in the admittedly conservative shoots, and the fact that the total number of parts is commonly a multiple of three in many Ranales, finds an explanation.

#### SUMMARY.

The meristic variation of the gynaecium in twelve hundred flowers of *Alisma plantago* is shown to range from nine to twenty-seven carpels with a primary mode of eighteen. Individual plants have modes of eighteen, twenty-one, and twenty, and the 'curve' shows evidence of a periodic character.

Data regarding the meristic variation of four hundred flowers of *Echinodorus ranunculoides* show marked periodicity both for the 'population' and the individual with a primary mode of thirty in normal plants and of twenty-four in the case of a dwarf race. Secondary modes of a pronounced character correspond to other multiples of three. The striking resemblance to the variation curve for the androecium of *Eranthis hyemalis* is emphasized. Examination of over a hundred flowers of *Sagittaria sagittifolia* shows a range in staminal number of from nine to thirty-four with modes of twenty-four, twenty-seven, and thirty. Detailed analysis of single inflorescences shows progressive increase in the number of carpels from below upwards. Abnormalities of the flower are described in which a single stamen replaces two paired stamens and vice versa.

Positive correlation is shown to obtain between the number of carpels and number of stamens in *Sagittaria*.

Proliferating flowers of *Sagittaria sagittifolia* suggest that the growing-point behaves as a multicellular equivalent of a three-sided apical cell.

Branched stamens and stamens bearing stipule-like appendages are described for *Sagittaria sagittifolia*.

In *Sagittaria obtusa* only a few flowers were examined, which showed from thirty to fifty-two stamens.

Flowers of *Butomus umbellatus* are comparatively stereotyped, but



flowers are described with supernumerary stamens, apparently due to fission, and with a single stamen replacing an antisepalous pair. The variation in number of stamens observed was eight to eleven, and of carpels four to six. Each carpel contains from 36 to 119 ovules, of which, however, many may abort.

Female flowers of *Stratiotes aloides* exhibit marked variation in the number and grouping of the staminodes, but these appear to form two whorls alternating with the perianth segments.

The perianth of *Butomus umbellatus* may show four members in the outer whorl, whilst an instance of fusion is described for that of *Stratiotes*. The data furnished with regard to the structure of the flowers considered are reviewed in comparison with other members of the group which it is suggested exhibit two chief tendencies, viz. towards centrifugal reduction and staminal fission. These, together with fusion of parts, appear to be responsible for the departures from the trimerous condition.

In Part II a comparison is instituted between the Ranales and the Helobiales, and in particular between the Ranunculaceae and the Alismataceae. In the light of the facts here given and the other resemblances between the two groups which are summarized, a closer affinity than is indicated in current classification should be recognized.

The plastic character of the two groups is emphasized and their retention of the trimerous construction discussed. The trimerous organization is regarded as characterizing the primitive type of Angiospermous flower from which the spiral type has been derived by mechanical displacement due to the large number of parts in relation to the rate of elongation of the floral axis. The primitive trimerous condition is attributed to the mode of division of the meristem, which latter is regarded as a multicellular derivative and equivalent of a three-sided apical cell.



# Studies in the Gramineae.

## I. The Flowers of certain Bambuseae.

BY

AGNES ARBER, M.A., D.Sc.

With eleven Figures in the Text.

### I. INTRODUCTION.

THE flowers of the Gramineae diverge so widely from the general Monocotyledonous type that even to-day there is no unanimity as to their interpretation. So it seemed to me that it might be of some use to make a comparative study of the spikelets by the method of serial sections, which has the advantage of disclosing the relations of the parts with an exactness which it is difficult to achieve in any other way. Schuster (12), some fifteen years ago, described various Grass flowers, of which he had cut microtome sections; I shall often have occasion to refer to his account in the course of these studies. The present instalment deals with the flowers of twenty species representing eight genera of Bamboos; I hope in later papers to describe other members of the Bambuseae, as well as various genera belonging to the Grasses in the narrower sense; to investigate certain abnormal forms; and to discuss the nature of the lodicules and other problems connected with the morphology of the flower and spikelet.

It is well known that many of the Bamboos produce their flowers only at long intervals of time (1, pp. 206-7), and the difficulty of obtaining material is thus very great. I wish here to express my gratitude to those whose kind help has made this study possible: the Director of the Jardin Botanique, Buitenzorg; the Director of the Royal Botanic Gardens, Kew; the Superintendent of the Royal Botanic Gardens, Sibpur, Calcutta; the Director of the Botanic Garden, Singapore; the Government Botanist, Department of Agriculture, Peradeniya, Ceylon; Mr. B. L. Gupta, Assistant Forest Botanist, Forest Research Institute, Dehra Dun; Mr. A. C. Hynes, Singapore; Mr. W. E. Kinsey, Assistant Conservator of Forests,

Federated Malay States; Mr. J. M. D. Mackenzie, Divisional Forest Officer, Katha Division, Burma; Mrs. Manners, Sua Gensing Estate, Rantau, F.M.S.; Mr. H. N. Ridley, F.R.S.; Professor B. Sahní, Lucknow; Professor A. C. Seward, F.R.S. Finally, I must record my special indebtedness to the late Mr. J. S. Gamble, F.R.S., who not only supplied me generously with material from his own magnificent collection, but was also most kind in identifying for me large numbers of specimens received from other sources.

## 2. METHODS.

Though I have used spirit material whenever available, I have been obliged to rely a good deal on herbarium specimens, and I have often found it possible to get serial sections from dried Bamboo spikelets by treating them with various modifications of McLean's method (6). The spikelets, after passing through 90 %, 60 %, 30 % alcohol, and then water, are treated with an 8 % to 9 % aqueous solution of caustic potash; after washing and neutralizing with 12 % to 15 % commercial acetic acid they are brought gradually up to absolute alcohol. I find that this method is not only applicable to herbarium specimens, but can be usefully employed for material preserved in spirit, if, as not infrequently happens in working with the Gramineae, it proves intractable. The necessary time in potash varies within wide limits; it depends partly upon the nature of the tissues, but it is much influenced by the temperature. I have used periods varying from a day to five weeks, but for herbarium material of average texture from one to seven days is commonly sufficient. The spikelets of the Bamboos, and indeed of the Gramineae in general, tend to offer some resistance to penetration with paraffin, and I have found it best to make the passage from xylol to paraffin a slow one; to allow at least a week, and often considerably more, in the paraffin bath; and to keep the latter at an unusually high temperature—about 59° C. when a wax melting at 51° C. is employed.

## 3. OBSERVATIONS.<sup>1</sup>

### Sub-tribe I: ARUNDINARIEAE.

#### *Arundinaria.*

Microtome sections of the flowers of *Arundinaria falcata*, Nees, have been figured by Rowlee (9) and of *A. Simoni*, A. and C. Rivière, by Schuster (12), so I have not attempted to examine the spikelets of this genus. I will only note that Rowlee's account of the morphology of the flower is confused by his statement that the 'palet' (palea) belongs to the same axis as the 'glume' (flowering glume); whereas in reality the

<sup>1</sup> The order in Bentham and Hooker's *Genera Plantarum* is followed in this section.

flowering glume is borne on the main axis—being the leaf (bract) in whose axil the flower arises, while the palea is the first leaf (bracteole) borne by the flower-axis itself.

*Arthrostylidium Schomburgkii*, Munro (Fig. 1).

The diagrams in Fig. 1 show the result of microtoming spikelets from a plant in the Cambridge Botany School Herbarium collected in British Guiana by R. H. Schomburgk, probably early in 1839 (11). It has been identified by Mr. J. S. Gamble as 'one of the few known specimens upon which the species *Arundinaria Schomburgkii*, Bennett—afterwards

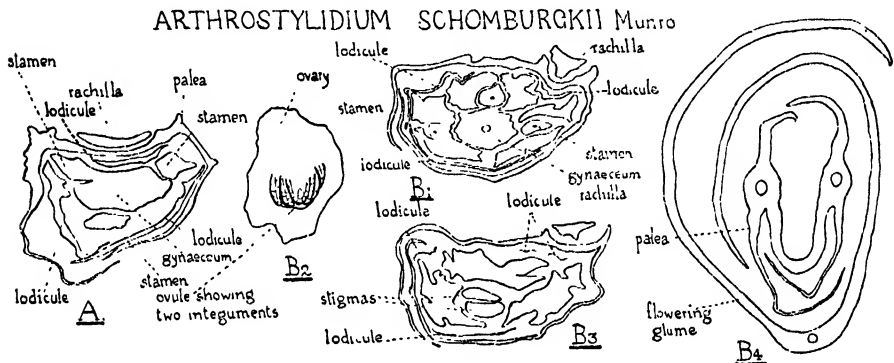


FIG. 1. *Arthrostylidium Schomburgkii*, Munro (*Arundinaria Schomburgkii*, Bennett), Cambridge Botany School Herbarium, collected by R. H. Schomburgk, British Guiana. A, transverse section low in flower, showing the third stamen just originating ( $\times 47$ ). B<sub>1</sub>–B<sub>4</sub>, transverse sections of one flower; B<sub>1</sub>, cut through the level of the anthers, and of the gynaeceum above the ovule ( $\times 47$ ). B<sub>2</sub>, ovary at a level slightly lower than B<sub>1</sub>, showing the ovule with two integuments ( $\times 77$ ); B<sub>3</sub>, cut at a higher level than B<sub>1</sub>, to show the two stigmas ( $\times 47$ ); B<sub>4</sub>, the flowering glume and palea at a level above the flower ( $\times 47$ ).

transferred to *Arthrostylidium* by Munro (Monogr., p. 41)—was founded'. I will not describe the sections in detail, since the herbarium material has suffered some distortion, and the general features of the Bamboo flower will be better understood from the drawings of *Bambusa* which come next. I will only here mention that the flower is of the three-stamened type, which is not found in the other sub-tribes. There are three lodicules (Fig. 1, A, B<sub>1</sub>, B<sub>3</sub>); the palea is markedly bikeeled in its upper region (Fig. 1, B<sub>4</sub>).

#### Sub-tribe II: EUBAMBUSEAE.

*Bambusa nutans*, Wall. (Fig. 2, p. 450).

From this species one can get a good general idea of the Bamboo flower. The bract or *flowering glume* borne by the spikelet-axis, or *rachilla*, has the flower in its axil (Fig. 2, A<sub>2</sub> and B). The bracteole or *palea* is bikeeled, and it is easily seen from the figures that this bikeeled form is an inevitable result of the compression which the young palea suffers

between the rachilla on the one side and the flower on the other. Passing to the flower itself, we find that the members of the outermost whorl—the three *lodicules*—are relatively large and richly vascular (Fig. 2, A<sub>2</sub> and A<sub>3</sub>). The two lateral lodicules are larger than the posterior one, and become free at a lower level. The filaments of the six stamens are shown

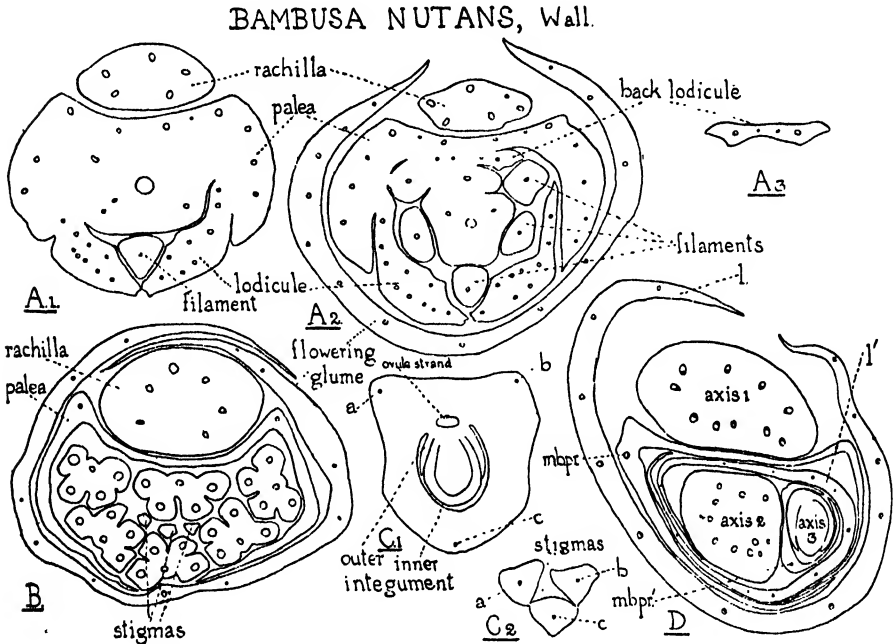


FIG. 2. *Bambusa nutans*, Wall. (from Lucknow; locality of collection unknown). A<sub>1</sub>–A<sub>3</sub>, transverse sections from series passing upwards through a flower ( $\times 77$ ); flowering glume, which was somewhat broken, reconstructed in A<sub>2</sub> and omitted in A<sub>1</sub>. A<sub>3</sub>, back lodicule after detachment. B, transverse section of another flower at a higher level ( $\times 77$ ) to show six anthers and three stigmas. C<sub>1</sub> and C<sub>2</sub>, transverse sections ( $\times 77$ ) through a very young gynaecium; C<sub>1</sub>, at the level of the ovule with its two integuments; C<sub>2</sub>, at the level of the separation of the stigmas; a, b, c, embryonic bundles passing into the stigmas. D, transverse section ( $\times 47$ ) of a small part of an inflorescence to show vegetative budding; axis 1, which is fertile at a higher level, bears at a lower level axis 2 and axis 3, which are sterile; m.b.pr., median bundle of prophyll borne on axis 2, which arises in the axil of leaf l. on axis 1 (leaf l. was slightly broken and has been reconstructed); m.b.pr', median bundle of prophyll borne on axis 3, which arises in the axil of leaf l' on axis 2.

in process of detachment in Fig. 2, A<sub>2</sub>, while the six anthers are cut in Fig. 2, B; the latter section is from a young flower in which the future mass of pollen grains is represented by a very small central group of sporogenous cells in each locus. The gynaecium is of a type very frequent in the Bamboos. Fig. 2, C<sub>1</sub>, shows the transverse section of the ovary. The ovule has two integuments, and is supplied by a vascular strand which runs up the ovary wall. There are three other vascular bundles in the gynaecium; a and b are lateral and posterior, while c is median and anterior. The gynaecium terminates above in three stigmas (Fig. 2, C<sub>2</sub> and B), into which

pass the three bundles *a*, *b*, and *c*. I propose to leave the question of the interpretation of the vascular system of the gynaecium until later in this paper (p. 465).

Fig. 2, D, illustrates a feature which is common in this species, and indeed in many Bamboos; this is the occurrence of vegetative buds, or sterile spikelets, in the inflorescence. In the example figured, *axis* 1 is fertile at a higher level, but *axis* 2 and *axis* 3 are purely vegetative.

*Bambusa Bambos*, Back. (Fig. 3, A-D, p. 452).

It is not necessary to describe *B. Bambos* in detail, as it closely resembles *B. nutans*. Sterile spikelets (*axis* 3 and *axis* 4) are seen in Fig. 3, A; *axis* 2, which has given rise to them, produces flowers at a higher level. Fig. 3, C, shows the close packing of the members which is so characteristic of the young Bamboo flower. In Fig. 3, B, the three lodicules are visible; at this level the back stamen is not yet detached from the gynaecium. Fig. 3, D<sub>1</sub> and D<sub>2</sub>, represent a teratological case in which two of the stamens are fused, the paired filaments bearing only six pollen-sacs, instead of the eight sacs which two free filaments would produce.

*Bambusa arundinacea*, Willd. (Fig. 3, E and F, p. 452).

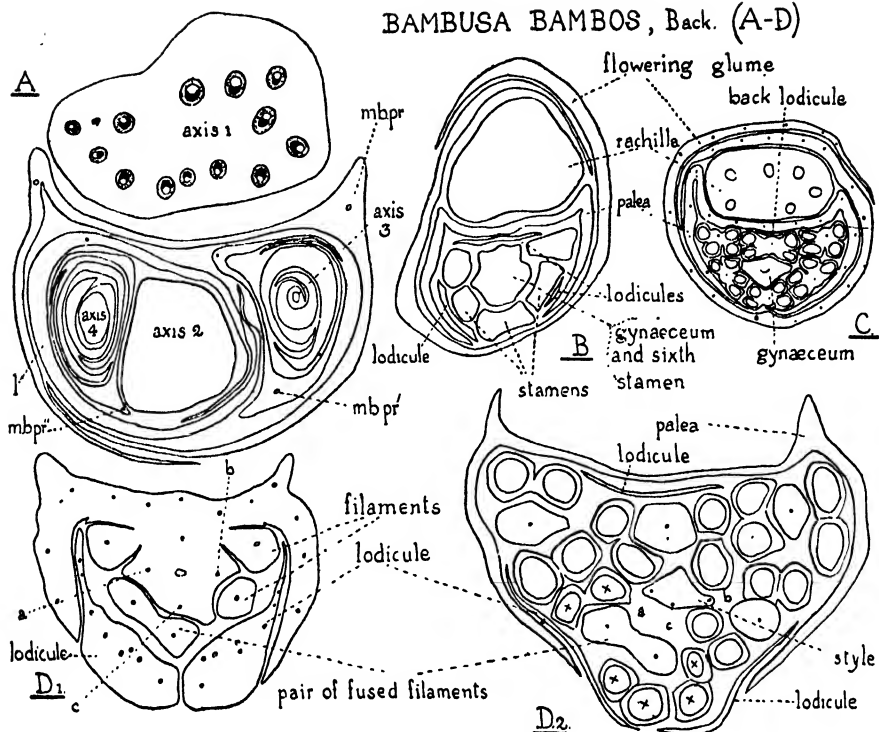
The sketches of *B. arundinacea* in Fig. 3 are given in order to illustrate two special characteristics of this species. In Fig. 3, E<sub>1</sub>-E<sub>4</sub> (a series taken from below upwards), we can follow the detachment of a flowering glume from the rachilla; its base extends downwards below the actual level of exertion as a fimbriated flap. Fig. 3, F, shows, on a larger scale, the flower which is borne by the rachilla drawn in Fig. 3, E. It will be seen that the front lodicules have a curious form; in section they may be compared to a fish-tail. I do not propose to do more now than to mention these two features of *B. arundinacea*, since they both recur in other Gramineae outside the Bamboos, and I hope in later papers to treat them comparatively.

*Gigantochloa Scortechinii*, Gamble (Fig. 4, A-C, p. 454).

The spikelet of *G. Scortechinii* contains several flowers, the terminal one being described as 'imperfect'. I microtomed three spikelets belonging to this species, and in each the terminal flower was represented by its axillant leaf—the flowering glume—alone (Fig. 4, A<sub>1</sub>, Flower 5). When the origin of this flowering glume is carefully followed in serial sections, it is found that it results from the transformation of the entire shoot-apex, no rudiment of a 'growing-point' being left over.

The flowers themselves differ in various points from those of the Bamboos hitherto considered. The most obvious divergences are that they

## BAMBUSA BAMBOS, Back. (A-D)



## BAMBUSA ARUNDINACEA, Willd. (E&amp;F)

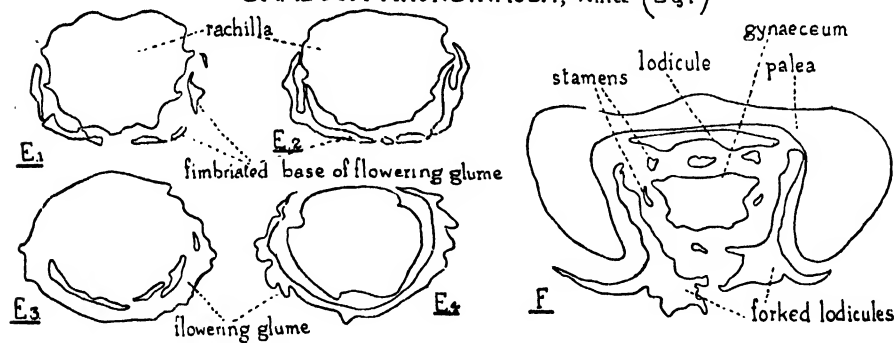


FIG. 3. *Bambusa*. A-D, *B. bambos*, Back. (from the Botanic Garden, Buitenzorg, Java). A, transverse section showing two sterile spikelets whose axes are marked *axis 3* and *axis 4* ( $\times 47$ ); *m.b.pr.*, probably the median bundle of the prophyll borne by *axis 2*, but reconstructed, as the keel was broken and the tissues displaced; *m.b.pr'*, median bundle of prophyll borne by *axis 3*, which arises in the axil of the prophyll of *axis 2*; *m.b.pr''*, median bundle of the prophyll borne by *axis 4*, which arises in the axil of leaf 1, which succeeds the prophyll of *axis 2*. B, transverse section near the base of a very young flower ( $\times 77$ ). C, transverse section of an older flower ( $\times 47$ ). D<sub>1</sub> and D<sub>2</sub>, transverse sections ( $\times 47$ ) at the base and at a higher level of a flower which is abnormal in having two stamens with their filaments fused; the pollen-sacs belonging to these two stamens are marked with crosses; *a*, *b*, *c*, gynaeceum bundles. In D<sub>2</sub> the vascular tissue is not shown in the palea, which is poorly preserved at this level. E-F, *B. arundinacea*, Willd. (from the Botanic Garden, Saharanpur, North India). E<sub>1</sub>-E<sub>4</sub>, series of transverse sections ( $\times 23$ ) passing upwards through the exsertion of a flowering glume to show fimbriated basal flap; E<sub>4</sub> is above the level of exsertion. F, transverse section of the flower of which the flowering glume is shown in E<sub>1</sub>-E<sub>4</sub> ( $\times 47$ ) to show the forked character of the front lodicules (recovery of herbarium material not perfect).



possess no lodicules, and that the filaments are united into a stamen-tube (Fig. 4, A<sub>1</sub>). A more interesting difference relates, however, to the vascular system of the gynaecium, which must be described in detail. The three spikelets (I, II, III), which I examined, each contained three or four functional flowers, followed by the reduced flower already described. I cannot say whether the spikelets were complete to the base, so the expression '*Flower 1*' in the following account means the basal flower in my preparations, but not necessarily the first flower of the spikelet. In the gynaecium of this species (and of various other Bamboos) there is a strand of small-celled tissue running up the centre of the style from the apex of the ovary cavity, and corresponding in position to the 'conducting tissue' of the styles of certain other Angiosperms; but as it is uncertain whether it represents the route by which the pollen-tube travels, I have used for it the non-committal name of 'stylar core' (Fig. 4, A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, B<sub>2</sub>, p. 454).

The vascular structure of the gynaecium in the different spikelets may be summarized as follows:

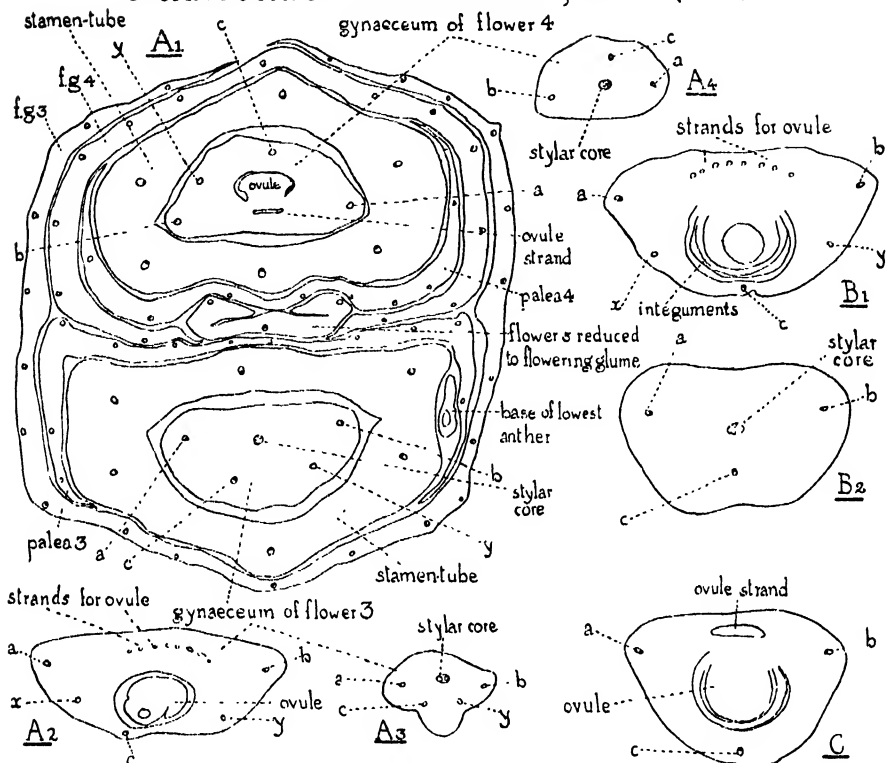
**Spikelet I** (three functional flowers). *Flower 1* has four bundles in the gynaecium at the level of the ovary—the placental bundle supplying the ovule and the three bundles (two lateral and one anterior) which I have called *a*, *b*, *c*; these three bundles pass into the style. This type of bundle system is illustrated in Fig. 4, C. *Flower 2* and *Flower 3* show the same four bundles as *Flower 1*, but, in addition, there is a fifth bundle between the anterior strand and one of the lateral bundles; this extra bundle dies out at the base of the style.

**Spikelet II** (four functional flowers). *Flower 1* and *Flower 2* are of the same type as **Spikelet I**, *Flower 1*. But *Flower 3* reaches a degree of complexity not attained in **Spikelet I**. At the level of the ovule (Fig. 4, A<sub>2</sub>) there is a non-lignified placental arc, as well as three lignified bundles (*a* and *b*, lateral and posterior, and *c*, anterior) which pass into the style. But in addition there is a non-lignified strand (*x*) between *a* and *c*, and another (*y*) between *b* and *c*. At the base of the style *x* dies out (Fig. 4, A<sub>1</sub>, *Flower 3*), but *y* enters the style, which is thus unusual in being four-bundled, at least at the base (Fig. 4, A<sub>3</sub>); I was unable to follow the structure to higher levels. *Flower 4* (upper flower in Fig. 4, A<sub>1</sub>) is of the five-bundled type—the fifth bundle, *y*, disappearing at the base of the style.

**Spikelet III** (three functional flowers). *Flower 1* belongs to the same type as the upper flower in Fig. 4, A<sub>1</sub>. *Flower 2* has six bundles at the level of the ovule-insertion (Fig. 4, B<sub>1</sub>). These are the lignified bundles, *a*, *b*, *c*; the placental bundle (represented by an arc of strands); and two unlignified bundles, *x* and *y*, belonging to the same whorl as the placental bundle.<sup>1</sup>

<sup>1</sup> For convenience the bundles *x* and *y* are here assumed to belong to an inner carpellary whorl, without prejudice to the discussion of the question on pp. 465–8.

## GIGANTOCHLOA SCORTECHINII, Gamble (A-C)



## GIGANTOCHLOA HETEROSTACHYA, Munro (D)

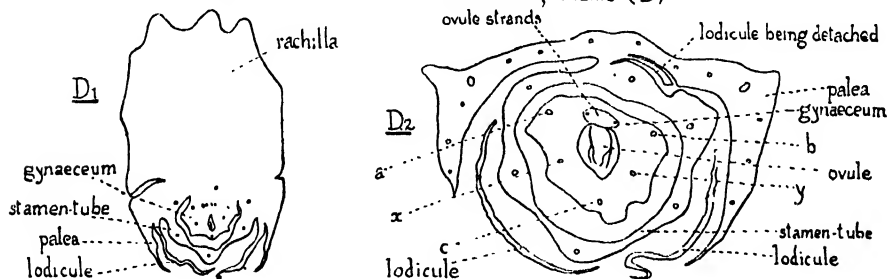


FIG. 4. *Gigantochloa*. Throughout the figure *a*, *b* (lateral), and *c* (anterior) are the bundles of the outer gynaecium whorl, while *x* and *y* are the lateral bundles of the inner gynaecium whorl, of which the ovule strand forms the posterior bundle. A-C, *G. Scortechinii*, Gamble (from near Bentong, State of Pahang, Federated Malay States). Drawings from transverse sections through spikelets (all  $\times 47$ ). A<sub>1</sub>, third, fourth, and fifth flowers of a spikelet; *fg.*, flowering glume. A<sub>2</sub>, gynaecium of third flower at a level lower than A<sub>1</sub>; A<sub>3</sub>, gynaecium of third flower at a level higher than A<sub>1</sub> and passing through base of style; A<sub>4</sub>, style of Flower 4. B<sub>1</sub> and B<sub>2</sub>, gynaecium from another spikelet; B<sub>1</sub>, ovary; B<sub>2</sub>, base of style. C, gynaecium of another flower belonging to the same spikelet as B<sub>1</sub> and B<sub>2</sub>. D<sub>1</sub> and D<sub>2</sub>, *G. heterostachya*, Munro (from Malacca, Lemann, 1845). D<sub>1</sub>, transverse section of a flower not completely detached from the rachilla ( $\times 23$ ); vascular strands omitted except in the stamen-tube and gynaecium. D<sub>2</sub>, transverse section at a higher level in the same flower on a larger scale ( $\times 47$ ); stamen-tube and lodicule on the right-hand side broken and slightly reconstructed.

At the base of the style the strands are reduced to three by the dying out of the placental bundle and of the two bundles belonging to the same whorl (Fig. 4, B<sub>2</sub>). Flower 3 is of the simple four-bundled type shown in Fig. 4, C.

From the details just given it follows that, in the 10 flowers examined, the gynaecium at the level of the ovule showed:

- 4 bundles in 4 flowers;
- 5 bundles in 4 flowers;
- 6 bundles in 2 flowers.

Each of the two six-bundled gynaecia belonged to a flower which was

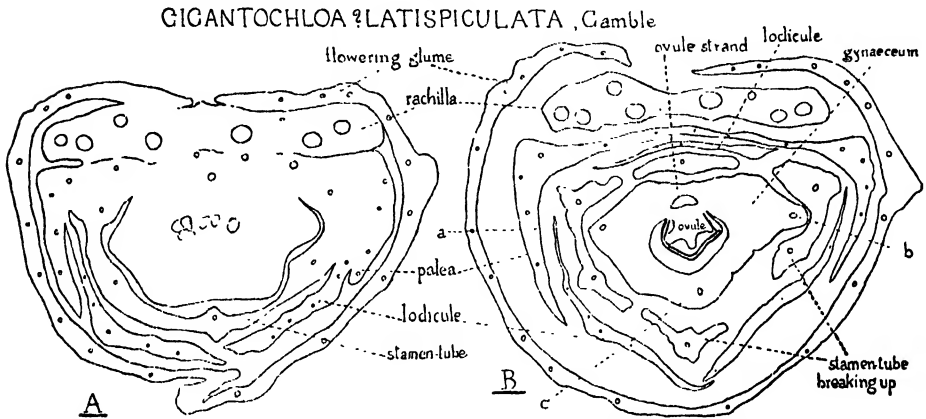


FIG. 5. Probably *Gigantochloa latispiculata*, Gamble (Jelebu District, N.S., Federated Malay States). A and B, transverse sections at the base and higher up through one flower ( $\times 43$ ).

neither the highest nor the lowest of the functional flowers in the spikelet; that is to say, it was probably in a position favourable to full development. In nine of the ten flowers, the three bundles of the outer whorl alone passed into the style; in the tenth flower one of the front bundles of the placental whorl also entered the base of the style.

The significance of the variable vascular system of the gynaecium of *G. Scortechinii* will be considered later (pp. 465-7).

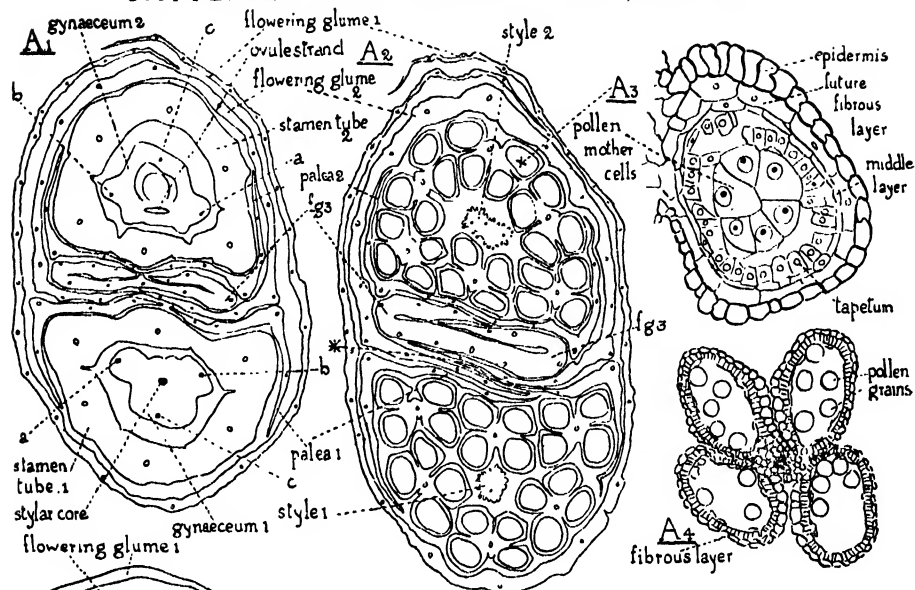
*Gigantochloa heterostachya*, Munro (Fig. 4, D, p. 454).

*G. heterostachya* differs from *G. Scortechinii* in having three lodicules (Fig. 4, D<sub>1</sub> and D<sub>2</sub>). I was able to make out the bundle system in only one ovary of the herbarium material which I examined, and here there were six bundles—*a*, *b*, *c*, *x*, *y*, and the placental bundle; the arrangement is thus identical with that in the ovary of *G. Scortechinii* drawn in Fig. 4, A<sub>2</sub>.

*Gigantochloa ?latispiculata*, Gamble (Fig. 5).

In sections of spikelets of a Malayan Bamboo, which is probably *G. latispiculata*, I have found three lodicules, as in *G. heterostachya*. In the

## OXYTENANTHERA NIGROCILIATA, Munro (A)



## OXYTENANTHERA ALBOCILIATA, Munro (B&amp;C)

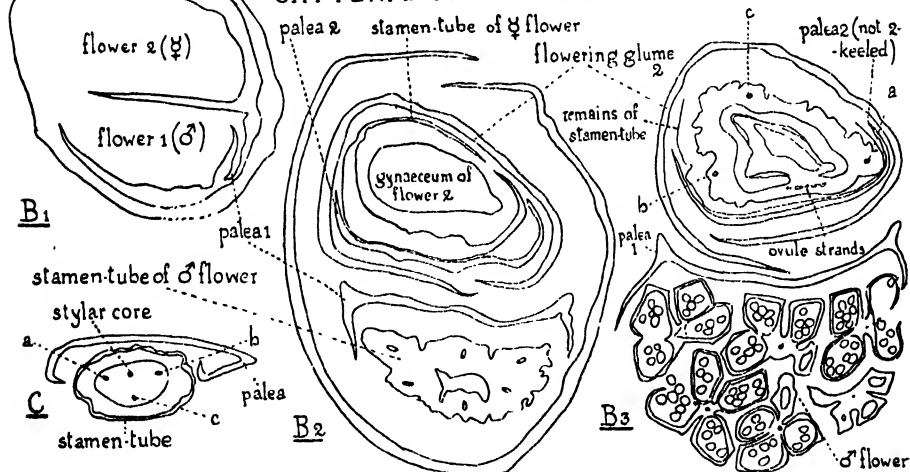


FIG. 6. *Oxytenanthera*. A, *O. nigrociliata*, Munro (from Perak, Malay Peninsula). A<sub>1</sub> and A<sub>2</sub>, transverse sections ( $\times 47$ ) through a spikelet cut at two levels, showing two functional flowers (1 and 2) and a third flower reduced to the flowering glume, fgs; a, b, c, the three bundles of the outer gynaeceum whorl. A<sub>3</sub>, one lobe of the anther marked with a cross in A<sub>2</sub> ( $\times 318$ ) from three serial sections. A<sub>4</sub>, transverse section of an anther from an older flower ( $\times 77$ ). B and C, *O. albociliata*, Munro (from Burma). B<sub>1</sub>-B<sub>3</sub>, transverse sections ( $\times 47$ ) through a spikelet with a male flower below and an hermaphrodite above; B<sub>1</sub>, cut at the base of the flower; B<sub>2</sub>, through the anthers of the male flower and ovary of the hermaphrodite flower. C, transverse section of another hermaphrodite flower to show the three-bundled style and the stamen-tube ( $\times 47$ ).

flower shown in Fig. 5, B, the stamen-tube begins to break up into separate filaments almost at the base of the androecium. The gynaecium is of the four-bundled type. In the extremely flat rachilla the epidermis and two or three of the hypodermal layers are strongly fibrous, and there is a fibrous sheath round each bundle.

*Oxytenanthera nigrociliata*, Munro (Fig. 6, A, p. 456).

In the spikelet of *O. nigrociliata* from Malay shown in Fig. 6, A<sub>1</sub>, we see two hermaphrodite flowers and a terminal 'flower', reduced to its axillant leaf, the flowering glume, *f.g.*<sub>3</sub>. A little below the section drawn this leaf was in the form of a closed leaf-sheath, but so completely flattened between the two preceding flowers that the cavity was a mere chink. There was no sign whatever of a vestigial apex at or above the exsertion of *f.g.*<sub>3</sub>, so that this leaf represents the transformation of the entire shoot apex. A second spikelet which I examined showed a similar terminal flowering glume.

*Flower 1* in Fig. 6, A<sub>1</sub> and A<sub>2</sub>, has a bimucronate palea, and it is noticeable that, at the asterisk in A<sub>2</sub>, one of the margins of the flowering glume of *Flower 2* is inserted between the two segments of the palea of *Flower 1*. The stamens are united into a tube as in *Gigantochloa*. The anthers, which are shown on a small scale in A<sub>2</sub> and in greater detail in A<sub>3</sub> and A<sub>4</sub>, are of the usual Grass type (4). The gynaecium is four-bundled (*gynaecium 2* in A<sub>1</sub>), three of the strands passing into the style (*gynaecium 1* in A<sub>1</sub> and *style 2* in A<sub>2</sub>).

*Oxytenanthera albociliata*, Munro (Fig. 6, B and C, p. 456).

I cut three spikelets of this species from Burma and found that the structure resembled that described by Schuster (12) for *O. abyssinica*, Munro, of Tropical Africa. Each spikelet is two-flowered. In two spikelets in which the history of both flowers could be followed, the lower was found to be male and the upper apparently hermaphrodite, though too old to show the anthers in my sections. Fig. 6, B<sub>1</sub>, is cut at the base of the two flowers. The palea of the lower flower is bikeeled, the wide separation of the keels being due to the fact that the flower is detached, not from a slender rachilla, but from the relatively solid mass which will at once differentiate into the second flowering glume with its axillary hermaphrodite flower. In this spikelet the mode of origin of the flowers can be followed completely, and it is certain that there is no rudiment of axis above the detachment of the flowering glume of the second flower, nor is there a terminal flower reduced to a flowering glume, as in *O. nigrociliata*. The result is that *Flower 2* is actually terminal, and, in correlation with this, the palea is not bikeeled as in *O. nigrociliata* (cf. *palea 2* in Fig. 6, B<sub>2</sub> and A<sub>1</sub>). Fig. 6, B<sub>2</sub>, shows the six-bundled stamen-tube of the male flower; at this level in the

hermaphrodite flower the stamen-tube is a mere remnant. The gynaeceum ( $B_3$ ) has three bundles ( $a, b, c$ ) in addition to the strands supplying the ovule.

Sub-tribe III: DENDROCALAMEAE.

*Dendrocalamus sikkimensis*, Gamble (Fig. 7, A, p. 459).

The general appearance of a small part of the inflorescence of this species is shown in Fig. 7,  $A_1$ , and a single two-flowered spikelet in greater detail in  $A_2$ . There are twelve stamens, six corresponding to each flower. The inflorescence was past maturity when it was drawn, and the anther sacs have emptied themselves through the pores indicated. Only one shrivelled style is visible. The gynaeceum is of the four-bundled type ( $A_3$ ) and the same is true of the two other species shown in Fig. 7 (*D. ?Hookeri* (B) and *D. Hamiltonii* (D)) and of the three species in Fig. 8, p. 461 (*D. strictus* (A), *D. giganteus* (C), and *D. membranaceus* (D)).

*Dendrocalamus ?Hookeri*, Munro (Fig. 7, B, p. 459).

Fig. 7, B, is a section through a three-flowered spikelet. *Flower 1* is male with a rudimentary gynaeceum which does not reach to this level; *Flower 2* and *Flower 3* are hermaphrodite. These sketches show the absence of lodicules which is generally characteristic of the genus. The bristle-like continuation of the rachilla above *Flower 3* is one-bundled; higher up it becomes sclerized and still more slender, but my sections do not reach to the point at which it actually dies out.

*Dendrocalamus Hamiltonii*, Nees et Arn. (Fig. 7, C and D, p. 459).

The spikelet drawn in Fig. 7, C, shows four flowers cut at different levels. *Flower 1* appears to be imperfect, consisting of four stamens only. *Flower 2* shows a gynaeceum and six stamens, but the section is cut too low to reveal all the anthers. *Flower 3* is cut at the level of the first appearance of the palea and of the three front stamens, while *Flower 4* is cut at the extreme base, where neither the flowering glume nor any member of the flower itself has become free.

*Dendrocalamus strictus*, Nees (Fig. 8, A and B, p. 461).

Fig. 8,  $A_1$ , which represents a transverse section through a spikelet, shows the four-bundled ovary in *Flower 2* and the three-bundled base of the style in *Flower 1*;  $a, b, c$  pass into the style, but not the ovule strand. The further history of the style-bundles can be followed in another gynaeceum, sections of which are sketched in  $B_1-B_5$ . The bundles  $a$  and  $b$  die out in passing up ( $B_3$ ) and the core of small-celled tissue which runs up the style from the apex of the ovary cavity is at length transformed into a hollow tube ( $B_4$ ), which finally opens to the exterior ( $B_5$ ). This is the only style



which I was able to follow to the tip, so I cannot say whether an open canal is a universal feature or not.

*Dendrocalamus giganteus*, Munro (Fig. 8, C, p. 461).

Fig. 8, C, shows a section of a very young flower of this species from Peradeniya. The front bundle, *c*, is the only lignified strand in the gynaeceum.

*Dendrocalamus membranaceus*, Munro (Fig. 8, D, p. 461).

Fig. 8, D, which is a section passing through a spikelet of this species from Burma, shows that its general flower structure, and the vascular system of the gynaeceum, are those characteristic of the genus.

*Teinostachyum attenuatum*, Munro (Fig. 8, E and F, p. 461).

In order to illustrate some member of the Dendrocalameae other than *Dendrocalamus*, I give here sketches showing sections of two flowers of *Teinostachyum attenuatum* from Ceylon. The herbarium material used was somewhat distorted, but one can make out the general features of the flower, which differs from *Dendrocalamus* in possessing three lodicules.

#### Sub-tribe IV: MELOCANNEAE.

*Schizostachyum brachycladum*, Kurz (Fig. 9, A-C, p. 462).

The general structure of the young flower of *S. brachycladum* is shown in Fig. 9, B<sub>1</sub> and B<sub>2</sub>. There are four bundles in the gynaeceum (A<sub>3</sub>), three of which pass into the style base (B<sub>2</sub>) and supply the three stigmas (B<sub>3</sub>). The three lodicules alternate as usual with the three outer stamens. In B these lodicules are of the familiar form, but in an older flower, sections of which are shown in A<sub>1</sub>-A<sub>3</sub>, the two front ones present a curious appearance. The palea at this stage has become sclerized, and between its firm lips emerge the anterior margins of the lodicules. At the base these margins are fused and form a projecting cushion, as if the soft tissue of the lodicules had been squeezed out to the exterior (A<sub>1</sub> and A<sub>2</sub>); a little higher they become free (A<sub>3</sub>). The flower shown in C<sub>1</sub> and C<sub>2</sub> is abnormal in having seven stamens, while in the anterior anther a flange of sterile tissue replaces one of the pollen-sacs. My sections also show two instances of unusual space-relations between the palea and other members. In B<sub>2</sub>, at the point marked with a cross, the margin of the flowering glume is inserted inside one margin of the palea—an appendage belonging to an axis of a higher order. This is not the case, however, at the extreme base, where the flowering glume is narrower. But at the cross in C<sub>1</sub> one margin of a lodicule is inserted between the margins of the palea—an arrangement which seems to hold good even to the very base, so that these members fail, from the beginning, to show their normal space-relations.



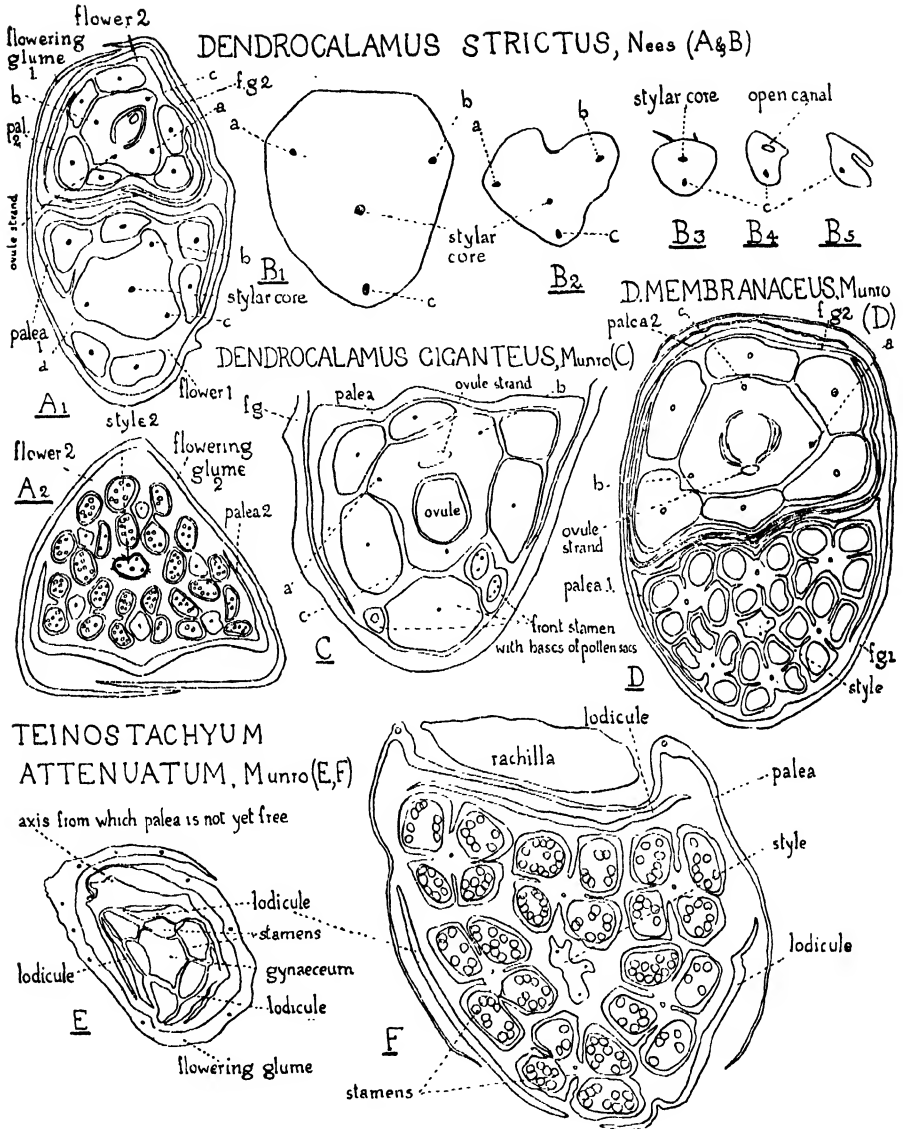


FIG. 8. A and B, *Dendrocalamus strictus*, Nees. A<sub>1</sub> and A<sub>2</sub>, transverse sections through a spikelet (from Dehra Dun) ( $\times 23$ ); vascular bundles omitted except in gynaecium and stamens. A<sub>1</sub> shows two successive flowers cut through their filaments; a and b, the two lateral, and c, the anterior bundle of the outer gynaecium whorl. A<sub>2</sub> shows the upper flower only, at a higher level, at which the anthers are visible. B<sub>1</sub>–B<sub>5</sub>, successive transverse sections of a style from a spikelet (from Bengal) showing the reduction from three bundles to one in passing up ( $\times 77$ ). C, *Dendrocalamus giganteus*, Munro. Transverse section of a flower from Peradeniya ( $\times 47$ ). The flowering glume, f.g., is incompletely shown. The section passes through the ovary and filaments, but the front stamen shows the base of an anther. D, *Dendrocalamus membranaceus*, Munro. Transverse section ( $\times 47$ ) showing two flowers (from Katha Division, Burma). Flower 1 is cut at the level of anthers and style, and flower 2 at the level of ovary and filaments. The flowering glume of flower 1 (f.g. 1) was slightly broken in the section and has been reconstructed. E and F, *Teinostachyum attenuatum*, Munro. Transverse sections of two flowers (from Ceylon) ( $\times 47$ ). E, close to extreme base; F, at level of anthers. The distortion is due to imperfect recovery of the herbarium material used.

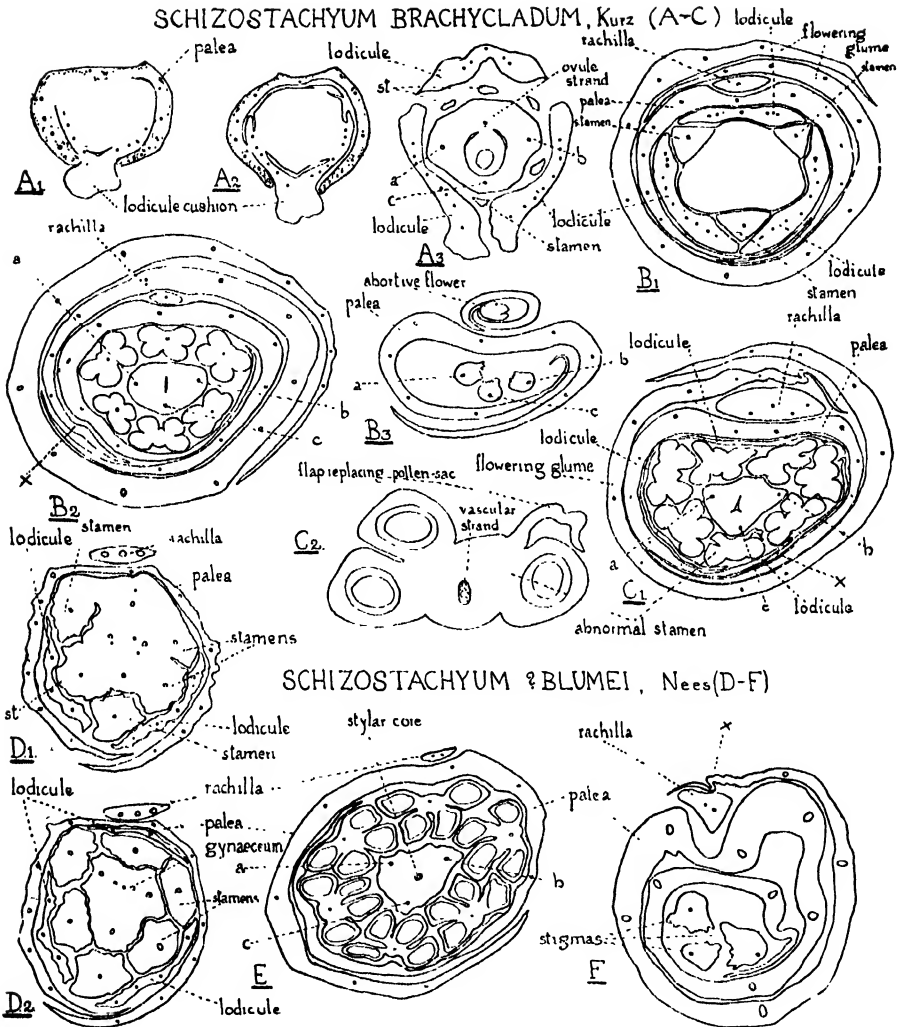


FIG. 9. *Schizostachyum*. A-C, *S. brachycladum*, Kurz (in cultivation at Singapore). A<sub>1</sub>-A<sub>3</sub>, sections from a transverse series through a flower whose stamens were exserted ( $\times 23$ ); a, b, c, bundles of outer gynaeceum whorl. B<sub>1</sub>-B<sub>3</sub>, sections from transverse series through a younger flower than A ( $\times 47$ ). B<sub>1</sub> is cut at a level at which the inner whorl of stamens is not yet detached; B<sub>2</sub>, at the top of the ovary where the cavity is represented by a chink, and above the level of the lodicules; B<sub>3</sub>, through the stigmas—flowering glume omitted. C<sub>1</sub> and C<sub>2</sub>, transverse sections of a flower from the same spikelet as B ( $\times 47$ ) passing through the gynaeceum just above the ovule; cavity of the ovary represented by a chink. The androecium is abnormal. C<sub>2</sub>, anterior stamen in C<sub>1</sub> ( $\times 193$ ). D-F, *S. ? Blumei*, Nees. D<sub>1</sub> and D<sub>2</sub>, transverse sections from a series through a flower ( $\times 47$ ); D<sub>1</sub>, at a level at which stamens and lodicules are not yet completely detached; D<sub>2</sub>, showing three lodicules and six stamens. E, transverse section of another flower ( $\times 77$ ); gynaeceum cut above ovule. F, transverse section of another flower passing through the stigmas ( $\times 77$ ).

*Schizostachyum* ?*Blumei*, Nees (Fig. 9, D-F, p. 462).

This plant, which was kindly sent me from the Botanical Garden at Buitenzorg, under the name of *Schizostachyum Blumei*, Nees, differs from the description of this species (e.g. that given by Gamble, 3, p. 116) in having three lodicules, so I have added a query to the specific name. The

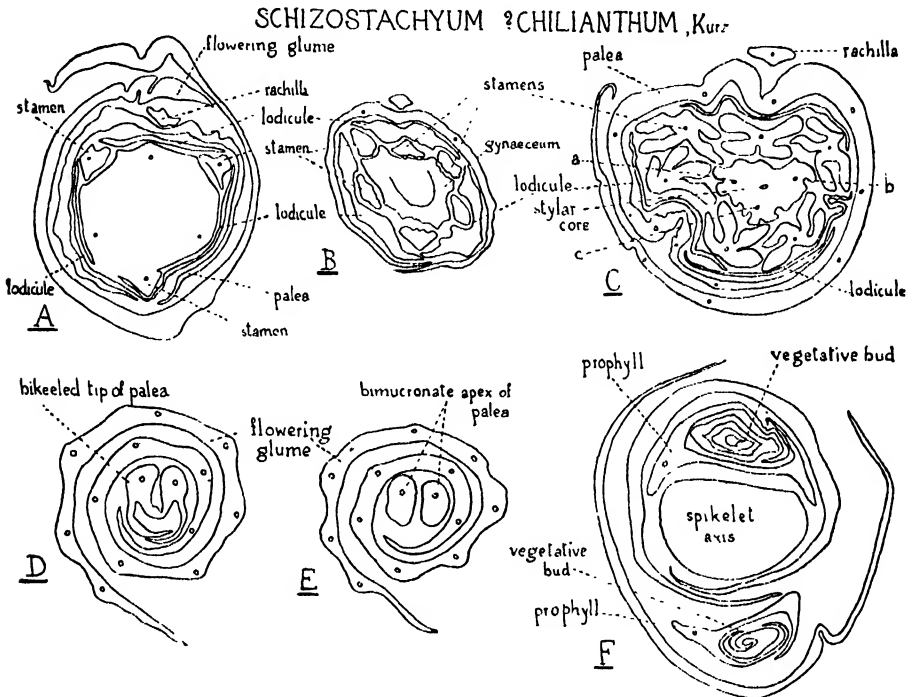


FIG. 10. Probably *Schizostachyum chilanthum*, Kurz (Jelebu District, N.S., Federated Malay States). All transverse sections,  $\times 47$ . A, base of a flower; vascular tissue shown only in stamens. B, near base of a flower (same spikelet as E) to show three lodicules and bases of stamens; flowering glume omitted. C, a flower at level of anthers; *a*, *b*, *c*, bundles of outer gynaeceum whorl; flowering glume omitted. D, near tip of spikelet passing through bikeeled palea. E, bimucronate termination of the palea whose lower region is shown in B. (In D and E the flowering glume was broken and has been somewhat reconstructed.) F, two vegetative buds occurring at the base of a fertile spikelet.

flower resembles *S. brachycladum* in general structure. It is noteworthy that at the cross in Fig. 9, F, the palea belonging to the secondary axis enwraps the rachilla—the primary axis.

*Schizostachyum* ?*chilanthum*, Kurz (Fig. 10).

This plant from Malay shows a general resemblance to the species sketched in Fig. 9. Its bikeeled and bimucronate palea is a striking feature (D and E). Vegetative buds occurring at the base of the spikelet are shown in F.

*Melocanna bambusoides*, Trin. (Fig. 11).

In the herbarium material of *M. bambusoides* which I microtomed only four flowers could be studied in detail. Each of these had five stamens; the genus is described as possessing five to seven stamens (3, p. 118). In

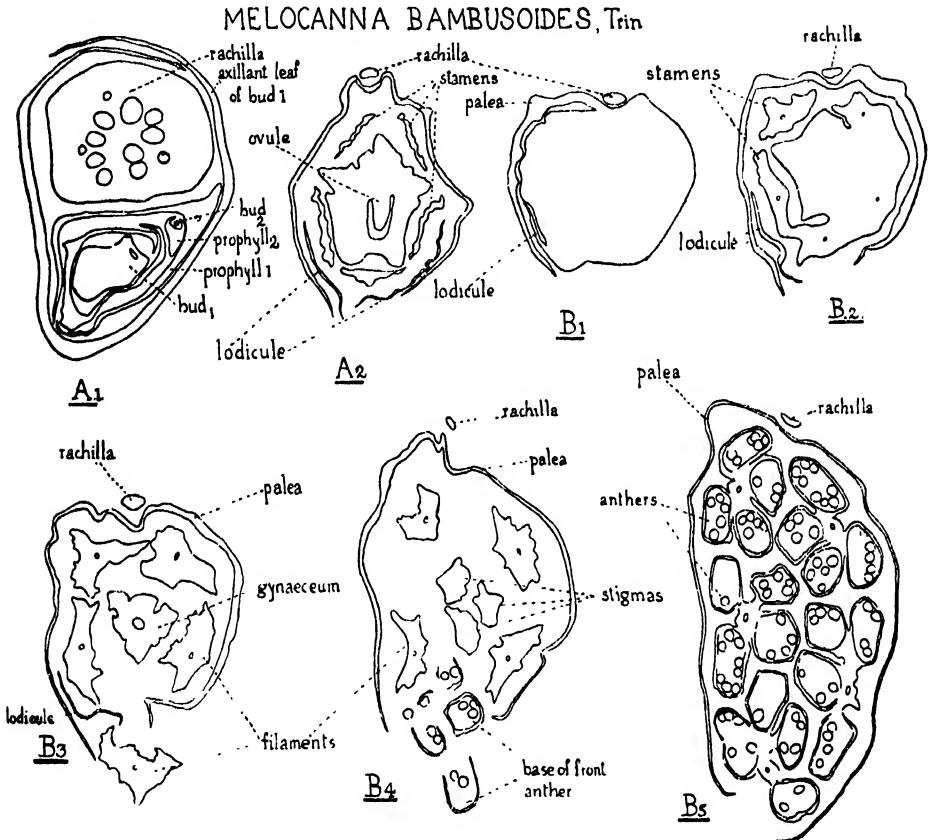


FIG. 11. *Melocanna bambusoides*, Trin. (Moulmein, Burma). A<sub>1</sub>, transverse section below the flower shown in A<sub>2</sub>. The vegetative bud 1 has bud 2 in the axil of its prophyll. A<sub>2</sub>, transverse section of the flower borne by the rachilla in A<sub>1</sub>. B<sub>1</sub>–B<sub>5</sub>, series of transverse sections through a flower with five stamens and one lodicule (all  $\times 47$ ). The distortion is due to imperfect recovery of the herbarium material used.

A<sub>1</sub> the spikelet has at its base a vegetative bud bearing a second bud in the axil of its prophyll. At a higher level the relatively massive rachilla shown in A<sub>1</sub> is almost entirely transformed into glumes and a flower, leaving only an extremely fragile continuation of itself (A<sub>2</sub>). The flower A has two lodicules, whereas B has only one. The three stigmas are shown in B<sub>4</sub>.

## 4. DISCUSSION.

We have now to consider the theoretical significance of a few points arising out of the observations here recorded; the discussion of a number of other questions—notably the interpretation of the lodicules—will be best deferred to later papers, in which I hope to supply some further evidence which cannot be included here.

(i) *Anomalous position-relations among members of the spikelet.*

It is usual in considering the morphology of the flower and its associated leaves to treat the way in which the margins of members overlap as evidence of their order of origin. But certain examples here figured show that this source of evidence must be used with caution. For instance, in a spikelet of *Oxytenanthera nigrociliata*, Munro, the margin of the flowering glume of one flower was found to be inserted between the two segments of the bimucronate palea belonging to the flower below (\* in Fig. 6, A<sub>2</sub>, p. 456). Again, in *Schizostachyum brachycladum*, Kurz, one of the margins of a flowering glume was found inside the edge of the palea, i.e. a leaf belonging to one shoot had one of its margins inside that of a leaf belonging to the next shoot (x in Fig. 9, B<sub>2</sub>, p. 462). Moreover, in *Schizostachyum ?Blumei*, Nees, a palea was seen to enwrap the rachilla of the spikelet, i.e. a leaf borne on a secondary axis enwrapped the primary axis (x in Fig. 9, F, p. 462). It should be noted that these three examples relate merely to marginal overlaps, which are the secondary result of growth in width on the part of organs whose bases are normally placed; I found one other instance, however, which gave evidence of more deep-seated disarrangement. This was in a flower of *Schizostachyum brachycladum*, Kurz, in which one margin of a lodicule lay outside one of the margins of the palea (x in Fig. 9, C<sub>1</sub>, p. 462). This grouping held good apparently to the base.

It is obvious that when—as in the Grasses—the leaves have broad bases which may more than encircle the axis from which they spring, and when the internodes are so reduced that a series of leaves may arise practically in one horizontal plane, the way is paved for such anomalies as I have described.

(ii) *The vascular system of the gynaeceum and its interpretation.*

The diagrams illustrating this paper bring out the fact that the commonest type of vascular system in the Bamboo ovary is that in which a dorsal strand supplies the ovule, while three other strands—one (c) ventral and opposite to the ovule-strand and two others (a, b), lateral and posterior—run up the ovary wall. This arrangement is sketched for *Gigantochloa ?latispiculata*, Gamble (Fig. 5, B, p. 455), *Oxytenanthera nigrociliata*, Munro

(Fig. 6, A<sub>1</sub>, gynaecium 2, p. 456), *O. albociliata*, Munro (Fig. 6, B<sub>3</sub>, upper flower, p. 456), *Dendrocalamus sikkimensis*, Gamble (Fig. 7, A<sub>3</sub>, p. 459), *D. strictus*, Nees (Fig. 8, A<sub>1</sub>, Flower 2, p. 461), *D. giganteus*, Munro (Fig. 8, C, p. 461), *D. membranaceus*, Munro (Fig. 8, D, upper flower, p. 461), and *Schizostachyum brachycladum*, Kurz (Fig. 9, A<sub>3</sub>, p. 462). The placental strand does not rise above the ovule, but the strands *a, b, c* pass into the base of the style; this will be seen in so many of the figures that it is unnecessary to refer to them individually—the lower flower in Fig. 6, A<sub>1</sub>, p. 456 (*Oxytenanthera nigrociliata*, Munro), will serve as an example. The central strand of small-celled tissue which I have called the 'stylar core' occupies the position of the 'conducting tissue' in the styles of various other Angiosperms; I think it has sometimes been mistaken for a non-lignified vascular bundle. I will not discuss it now, as I hope to return to its study in a later paper. In species in which there are three stigmas, each of the strands, *a, b, c*, passes into one of the stigmas, e.g. *Bambusa nutans*, Wall. (Fig. 2, C<sub>1</sub> and C<sub>2</sub>, p. 450), *Schizostachyum brachycladum*, Kurz (Fig. 9, B<sub>2</sub> and B<sub>3</sub>, p. 462), and *S. ?Blumei*, Nees (Fig. 9, E and F, p. 462).

The orthodox view (e.g. that taken by Hackel in Engler and Prantl's 'Pflanzenfamilien', 5, p. 8) is to regard the Grass gynaecium as monocarpellary. But the occurrence of the type of vascular symmetry just described has led Schuster (12, Fig. 32, p. 254) to return to the view of Čelakovský (2) and others, that the gynaecium of the Grasses is tricarpellary—the bundles *a, b, c* being the midribs of the carpels, and the single ovule being borne on the suture between the two posterior carpels. A third view, which again increases the number of carpels, has recently been put forward by E. R. Saunders (10, p. 155). This author holds that the number of carpels in many Angiosperms has been underestimated in the past, owing to the failure to recognize the existence of sterile as well as fertile carpels in the gynaecium. It seems probable from her work that in the Monocotyledons the typical gynaecium consists of two alternating whorls, each of three carpels, rather than one whorl of three carpels, as has been hitherto held. Saunders applies this interpretation to the Gramineae and concludes that the bundles which I have called *a, b, c* are the midribs of the outer whorls of carpels, while the ovule strand is the midrib of the posterior member of the inner whorl, *the two anterior members of the inner whorl having been reduced to such a point as to lose not only their external individuality, but even their vascular strands*.

In studying the gynaecium of the Bamboos, I had in mind the possibility that—assuming the validity of the theory just outlined—one might come on some member of the group in which the anterior members of the inner gynaecium whorl had survived in some degree. And this expectation has been realized in the genus *Gigantochloa*. I have fully described the vascular system of the pistil of *G. Scortechinii*, Gamble (p. 451), and I will

not here repeat the details, but reference to Fig. 4, p. 454, will show that in this species we not only find gynaecea of the familiar four-bundled type, but also others in which two additional bundles,  $x$  and  $y$ , are present in the anterior and lateral positions (Fig. 4,  $A_2$  and  $B_1$ ). In another species, *G. heterostachya*, Munro, the only flower whose structure I was able to follow, again showed a six-bundled gynaeceum (Fig. 4,  $D_2$ , p. 454). The bundles  $x$  and  $y$  in these two species occupy the positions postulated by Saunders for the two front carpels of the inner whorl; moreover, their inconstant mode of occurrence in *G. Scortechinii* is just what might be expected of vestigial structures. The vascular system thus harmonizes completely with the requirements of the polymorphic carpel theory, and on this theory the flower of *Gigantochloa heterostachya* might be taken to represent the fullest known development of the whorls which constitute the Grass flower, since there are 3 lodicules, 3 + 3 stamens, and 3 + 3 carpels. But it must at the same time be admitted that the fact of the existence of the bundles  $x$  and  $y$  does not in itself amount to a *proof* of the six-carpel theory, since it is certainly possible to regard the ovule strand and the bundles  $x$  and  $y$ , not as the midribs of an inner carpellary whorl, but as originating by fusion of the marginal bundles belonging to the three carpels whose midribs are the bundles  $a$ ,  $b$ , and  $c$ ; the vascular scheme would then be analogous to that of those Labiate calyces in which five sepals are responsible for ten bundles. I think, however, that the balance of probability favours Saunders's view that the Grass gynaeceum is six-carpellary, and that this view may be accepted as a working hypothesis for the family, so long as we do not lose sight of the fact that it is not yet proven.

(iii) *The occurrence of a terminal leaf in certain spikelets.*

Setting aside the controversial case of the phylloclades of the Rusceae, the only record in the literature of a vegetative leaf which is entirely terminal seems to be that of Queva (8), who describes the shoot of *Uvularia grandiflora*, Sm., as ending in a leaf and showing a complete absence of a 'cône végétatif terminal'. But the validity of this record seems open to doubt, since Queva does not appear to have used serial sections, or to have examined a shoot whose development was completed. The extremest example which I have myself studied was an unidentified species of *Uvularia*, in which I found the apex of the vegetative shoot reduced to a minute non-vascular rudiment, which never became free from the ventral surface of the base of the uppermost leaf (1, Fig. xxxv, 5, p. 58). The observations described in the present paper show, however, that among Bamboo spikelets we may find leaves (bracts) whose completely terminal character is clearly demonstrable. In *Gigantochloa Scortechinii*, Gamble, and in *Oxytenanthera nigrociliata*, Munro, I have found that the 'imperfect flower' in which the spikelet terminates may be reduced to its axillant leaf

—the flowering glume—alone (Fig. 4, A<sub>1</sub>, *Flower* 5, p. 454, and Fig. 6, A<sub>1</sub> and A<sub>2</sub>, *fg.* 3, p. 456). Moreover, serial sections prove that the entire shoot apex is transformed into this leaf, no tissue whatever being left over to form the rudiment of a 'growing-point'.

## 5. SUMMARY.

### (i) *Observations* (pp. 448–64).

The spikelets of the following Bamboos are described and illustrated :

Sub-tribe I: ARUNDINARIEAE.—*Arthrostylidium Schomburgkii*, Munro (p. 449 and Fig. 1, p. 449).

Sub-tribe II: EUBAMBUSEAE.—*Bambusa nutans*, Wall. (p. 449 and Fig. 2, p. 450); *B. Bambos*, Back. (p. 451 and Fig. 3, A–D, p. 452); *B. arundinacea*, Willd. (p. 451 and Fig. 3, E and F, p. 452); *Gigantochloa Scortechinii*, Gamble (p. 451 and Fig. 4, A–C, p. 454); *G. heterostachya*, Munro (p. 455 and Fig. 4, D, p. 454); *G. ?latispiculata*, Gamble (p. 455 and Fig. 5, p. 455); *Oxytenanthera nigrociliata*, Munro (p. 457 and Fig. 6, A, p. 456); *O. albociliata*, Munro (p. 457 and Fig. 6, B and C, p. 456).

Sub-tribe III: DENDROCALAMEAE.—*Dendrocalamus sikkimensis*, Gamble (p. 458 and Fig. 7, A, p. 459); *D. ?Hookeri*, Munro (p. 458 and Fig. 7, B, p. 459); *D. Hamiltonii*, Nees et Arn. (p. 458 and Fig. 7, C and D, p. 459); *D. strictus*, Nees (p. 458 and Fig. 8, A and B, p. 461); *D. giganteus*, Munro (p. 460 and Fig. 8, C, p. 461); *D. membranaceus*, Munro (p. 460 and Fig. 8, D, p. 461); *Teinostachyum attenuatum*, Munro (p. 460 and Fig. 8, E and F, p. 461).

Sub-tribe IV: MELOCANNEAE.—*Schizostachyum brachycladum*, Kurz (p. 460 and Fig. 9, A–C, p. 462); *S. ?Blumei*, Nees (p. 463 and Fig. 9, D–F, p. 462); *S. ?chilianthum*, Kurz (p. 463 and Fig. 10, p. 463); *Melocanna bambusoides*, Trin. (p. 464 and Fig. 11, p. 464).

### (ii) *Discussion* (pp. 465–8).

Attention is drawn to a few anomalous position-relations among the leaves of the spikelets described, and it is shown that the way in which the margins of members overlap is not always a safe criterion for the order of their origin (p. 465).

The vascular system of the gynaeceum is then discussed (pp. 465–7) and it is recorded that, in addition to the four-bundled type of gynaeceum which seems to be usual among the Bamboos, we may get in *Gigantochloa* a six-bundled type whose structure is in harmony with the requirements of the polymorphic carpel theory (10), according to which the Grass gynaeceum is six-carpellary. It is held, however, that this theory is



not at present proved for the Gramineae, though the balance of probability is in its favour.

Finally (pp. 467–8), it is shown that a leaf completely terminal to a shoot may occur in the spikelets of *Gigantochloa* and *Oxytenanthera*.

BALFOUR LABORATORY,  
CAMBRIDGE.  
October 26, 1925.

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# The Morphology of *Claustula Fischeri*, gen. et sp. nov. A New Genus of Phalloid Affinity.

BY

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With Plate XV and six Figures in the Text.

WHILE collecting fungi in August 1923 on one of the hills in the vicinity of Nelson (South Island, New Zealand), a group of what seemed to be an unusual type of Phalloid was found by the writer. The specimens were growing at a height of 1,500 ft. above sea-level, near the edge of a path, among moss and practically under tea tree (*Leptospermum scoparium*, Forst.).

The fungus consists of a volva and a globose to ovate receptaculum. When mature the receptaculum partially emerges from the enclosing volva, but its lower half remains loosely held within the latter by the lobes into which it has split (Pl. XV, Fig. 1). In the largest specimen found, in which the receptaculum was in its final position, the length from the free end of the receptaculum to the base of the volva was  $2\frac{1}{2}$  in., while the width of the receptaculum was 2 in. The volva is ovate before rupture, fleshy-firm in texture, and when undisturbed in the soil is white, but on exposure to the air becomes a reddish brown. When mature it ruptures into usually five pointed segments whose length is  $\frac{1}{3}$  to  $\frac{1}{2}$  that of the whole volva. The segments do not become reflexed, but tend to lie in the position they occupied before cleavage, with the result that they rest lightly upon the receptaculum from its equatorial region downwards, and so prevent it from emerging completely. The volva is composed of two layers, the outer of which is a firm, thin, finally coloured pellicle, while the inner is a wider, gelatinous, white layer, slippery to the touch, and thin above where the segments are formed, but gradually increasing in thickness in the equatorial and basal regions, where a width of 1.5 to 2 mm. is attained (Pl. XV, Fig. 2). The volva narrows at the extreme base and through it at this point there passes up from the exterior a slender, fibrous strand about 2.5 mm. wide and 7 mm.

high. The upper end of this strand is attached to the base of the receptaculum and the lower end is doubtless continued into a rhizomorph, although this was not secured with the specimens. As the receptaculum enlarges at maturity and is forced up the volva by the swelling of the gelatinous inner layer of the latter, it breaks away from this strand and thenceforth has a small hole at its base where the strand was originally attached. The strand, though small, remains visible even in old specimens and can be seen transversing and projecting a few millimetres within the gelatinous layer of the volva.

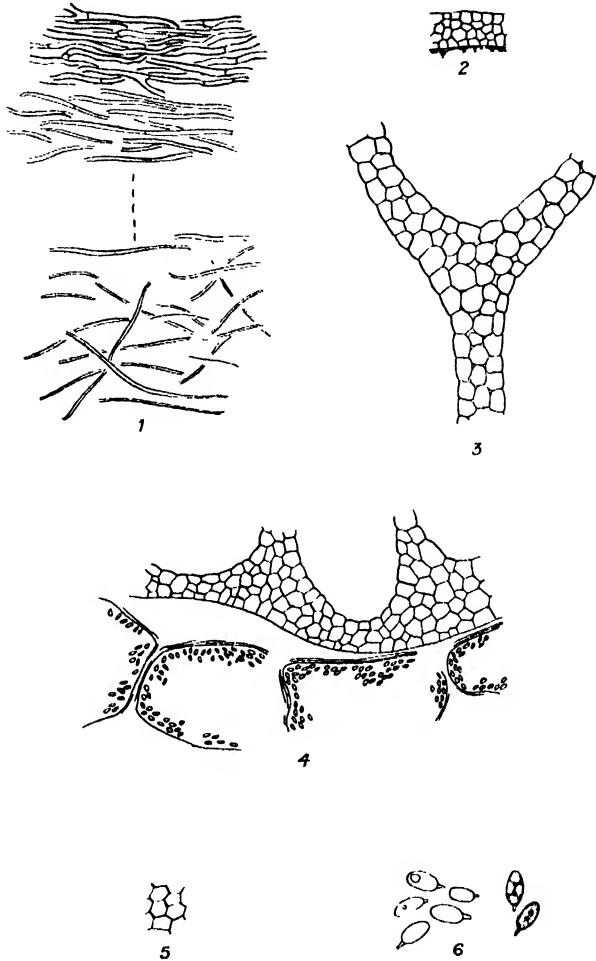
The receptaculum is simply a smooth white shell with a large central cavity. As already mentioned, it may be 2 in. across, and as the wall is only from 5 to 8 mm. thick, the diameter of the central cavity is relatively enormous. The structure of the wall is noteworthy. It consists entirely of meshwork, the strands of which are delicate except where several join, and the rounded cavities of which, though ranging in size, are usually large, often attaining a diameter of more than a millimeter (Text-fig. 4).

The hymenium is arranged in a layer of small chambers extending over the inner surface of the receptaculum, and therefore in contact with the cavity of the interior. When mature the walls of the hymenial chambers rupture, and the spores and the disintegrating hymenial and subhymenial tissues together form an intermittent and at times jagged layer over the inner surface of the receptaculum (Text-fig. 4).

#### MORPHOLOGY.

*Volva.* The outer surface of the pellicle consists of a dense aggregation of hyphae which in the unexposed volva when buried in the soil are hyaline, but which become a light brown, when viewed in section, when the volva has been exposed to the air. These hyphae are sparingly septate and branch infrequently at a wide angle. The width of the branch-hyphae is almost equal to that of the parent hyphae from which they arise. They are about  $2\ \mu$  wide and the greater part of this width is occupied by the lumen. The free ends of the hyphae are sometimes irregularly upturned, causing a minute roughness on the surface of the volva. Such colour as is present is confined to the hyphae at the surface, those below being hyaline. The sub-epidermal hyphae, although belonging to the pellicle, exhibit transitional stages not only in loss of colour but in the change from the parallel arrangement characteristic of the surface layers to the loose web which occurs throughout the inner gelatinous layer. In addition, branching becomes less frequent in the inner part of the pellicle, and the width of the hyphae is reduced, until finally it is no greater than that of the extremely slender hyphae characteristic of the gelatinous layer. In the latter region they are rarely branched and are distributed throughout the gelatinous matrix seem-

ingly indiscriminately as far as direction is concerned, but evenly and sparsely with regard to one another (Text-fig. 3). There is no differentiation into a membrane on the inner surface of the volva, matrix and hyphae merely



TEXT-FIGS. 1-6. 1. Volva, transverse section showing pellicle and mucilaginous layers.  $\times 440$ . 2. Receptaculum, transverse section.  $\times 1\frac{1}{2}$ . 3. Mesh of receptaculum.  $\times 440$ . 4. Hymenial chambers on inner surface of receptaculum.  $\times 440$ . 5. Surface view of layer covering hymenial chamber.  $\times 440$ . 6. Spores, surface view, and stained.  $\times 560$ .

ending abruptly in a smooth, slippery surface, the hyphae of which show no closer aggregation than is to be found deeper in the layer.

**Receptaculum.** The wall of the receptaculum is usually three to four chambers wide, but if the latter are small they are correspondingly more numerous (Text-fig. 4). The outline of the chambers is spherical, oval, or even angular, but with the angles rounded, never sharp. The mesh of the

wall consists of extremely thin-walled, usually spherical cells which are peculiarly uniform in size. The strand at its narrowest part, i.e. where it lies between two cavities, is three cells wide as a rule, but it is not unusual for there to be only two cells, and on rare occasions single-celled strands have been observed. At the junction of several strands, on the other hand, the number of cells is increased and may be as many as twenty (Text-fig. 5). No thickening of the cell-walls, nor closer aggregation, occurs at either surface of the receptaculum, all cells alike, from one surface to the other, being characterized by extreme delicacy, uniformity of size, and high water-content.

The hymenial cavities are more or less lenticular in shape and are arranged edge to edge with their longer axis in the plane of the surface of the receptaculum (Text-fig. 4). There is at times only a single row of sterile cells separating the hymenial chambers, but the width of this intervening tissue is not uniform and it may even exceed that of the chambers. The width of the chambers varies from  $100\mu$  to  $250\mu$ , while the height is not usually greater than  $100\mu$ . Even the smallest chambers seldom contain less than 100 spores and in the larger the number is nearer 1,000. The spores are borne over the whole surface of the chamber—on the inner surface of the covering wall as well as on the surface adjoining the receptaculum. Fragments of the wall covering the chambers were secured, and sections and surface views of them obtained (Text-fig. 5). The cells of these walls are angular rather than rounded, and smaller than those of the receptaculum generally. The wall in this part seems not to have been more than three cells wide and in some instances was only that of a single cell (i.e. in addition to the hymenium and subhymenium).

All the specimens obtained were mature, and basidia were no longer to be found. Moreover, internal decay sets in early in these fruit-bodies, and the structure of even the subhymenium could not be followed in any of the specimens. The conjugate nature of the nuclei, however, is still evident even in cells which have collapsed. The spores are brown in the mass but yellow with a slight fuliginous tone in transmitted light. In shape they are elliptical to rounded-elliptical, and are either symmetrical or have one side less curved than the other. As a rule, however, they are symmetrical and vary in size from  $8\mu$  to  $13\mu \times 5\mu$  to  $6\mu$  (Text-fig. 6). Both ends are rounded, but the free end may be slightly narrower than the base. The wall is smooth and the contents are clear, while the protoplasm is slight and the vacuoles relatively large. The spore is binucleate, the two nuclei lying as a rule side by side in a small cytoplasmic aggregation suspended in the centre of the spore by extremely delicate strands. It frequently happens that both nuclei lie in the plane of the longer axis of the spore, but this is not invariable. The only remarkable feature that the spores exhibit is the presence at their lower end of a persistent, stout, hyaline, obconic papilla (sterigma). This papilla

is about  $1.5\ \mu$  long and  $0.75\ \mu$  wide at its widest part, which is at its point of attachment to the spore.

The fungus is either an unusual member of the Phallineae or has affinities with that group. In the absence of material sufficiently young to trace its early development, its systematic position cannot be fully determined. It shows greatest resemblances in form to those members of the genus *Clathrus*, segregated by Lloyd as *Laternea*, in which the divisions of the receptaculum are few in number and united at their apex. But so far there is no recorded case in which a species of *Clathrus* has failed to exhibit any divisions. Moreover, the cellular structure of the receptaculum of *Claustula* resembles that of the stalk of certain species of *Simblum*, *Anthurus*, *Colus*, *Aseroë*, and *Phallus*, in which the cells form a pseudoparenchyma, rather than that of the receptaculum of *Clathrus*, where the cells on the whole are tubular. If one could conceive a *Simblum* or a *Colus*, or even an *Aseroë*, in which the gleba is located within the hollow stem instead of at the top of it, and developed generally over the interior instead of being confined to definite areas, and which, moreover, either has not yet evolved or possibly has lost the power of developing any of the various methods of exposing the spores characteristic of these genera, then the organism so conceived would not be unlike that at present under discussion. Both *Anthurus* and *Colus* include species, with few arms in the receptaculum, in which variation in the number of the arms is common. A form lacking arms would therefore not greatly differ from some of the simpler types of these genera already known.

Again, the genus *Sphaerobolus* exhibits some resemblance to the present genus. The receptaculum is more complex, being composed of three layers instead of one as in *Claustula*, but of these three layers the outer and part at least of the inner are similar in their pseudoparenchymatous nature to the receptaculum of *Claustula*. The gleba of *Sphaerobolus*, on the other hand, fills the space within the receptaculum instead of lining its inner surface. Nevertheless, there is a certain resemblance in the grosser features of the two genera. The spores, moreover, are more clearly of the shape and size of those of *Sphaerobolus* than of the small spores characteristic of the Phallineae. A further characteristic in which *Claustula* differs from members of the Phallineae is in the absence of gluten from the spores, for in the present genus they are only slightly viscid to the touch.

In the absence of details of the early development of *Claustula* speculation as to its relationships with other genera must necessarily be hazardous, and it is therefore hoped that early stages will be found, so that its systematic position may be established.

# DIAGNOSIS.

*Claustula*, gen. nov. Volva ovate, up to 2 in. in diameter, white at first, finally coloured, of two layers, outer thin, inner gelatinous, rupturing above at maturity into several pointed segments; receptaculum globose, permanently closed, partly ejected from volva at maturity, with large central cavity and wall of meshwork; gleba in small chambers on inner surface of receptaculum; spores brown, smooth, cylindrical with persistent basal papilla.

*Claustula Fischeri*, gen. et sp. nov. Volva ovate, about  $1\frac{3}{4}$  in. in diameter, at first white, then reddish brown, outer layer thin, coloured, inner layer wide, gelatinous, white; splitting above into usually five pointed lobes.

Receptaculum globose, white, smooth, up to 2 in. long, permanently closed, originally attached at base by fine strand to volva, ejected at maturity but held by lobes of volva; wall of receptaculum three-four chambers wide, mesh between chambers usually three cells wide, but may be twenty at the angles; cells thin-walled, more or less spherical, uniform in size, forming a pseudoparenchyma; hymenial chambers about  $200\mu \times 100\mu$ , in a single layer over the inner surface of receptaculum; spores brown in mass, fuliginous-yellow in transmitted light, elliptical, smooth,  $8\mu$  to  $13\mu \times 5\mu$  to  $6\mu$ , with basal papilla  $1.5\mu \times 0.75\mu$ .

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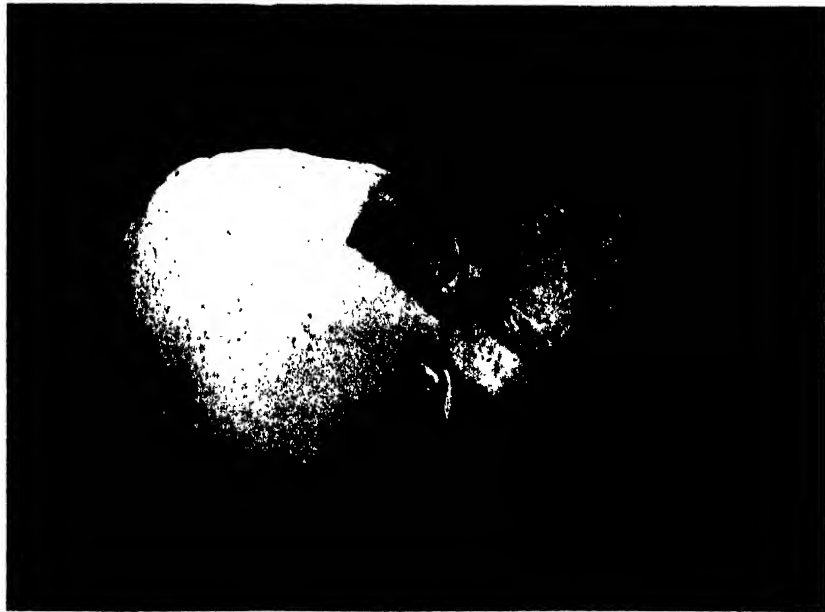
EXPLANATION OF PLATE XV.

Illustrating Dr. K. M. Curtis's paper on *Claustula Fischeri*.

Fig. 1. *Claustula Fischeri*, surface view.  $\times 1\frac{1}{2}$ . Photograph by W. C. Davies.

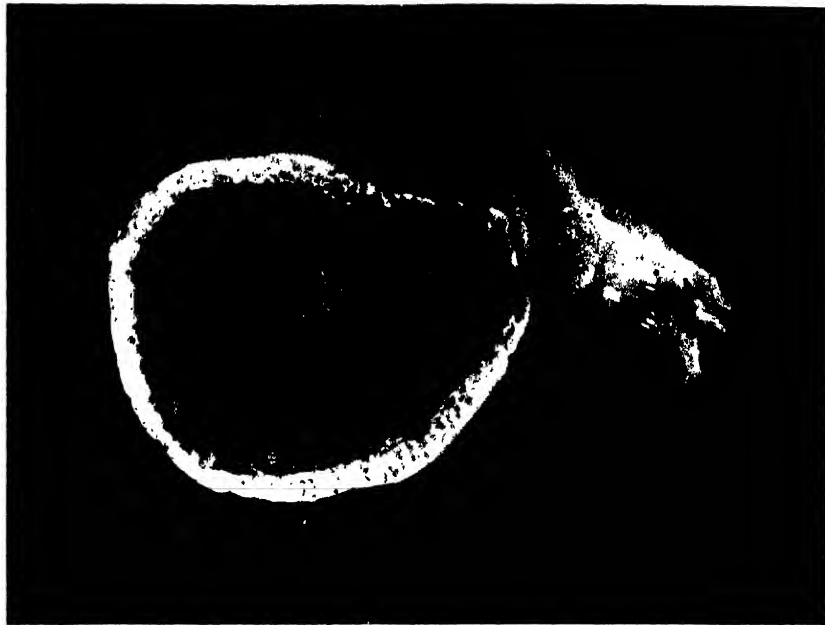
Fig. 2. *Claustula Fischeri*, in longitudinal section.  $\times 1\frac{1}{2}$ . Photograph by W. C. Davies.





W.C. Davies photo.

1



Hutchins.

2.

CURTIS-CLAUSTULA FISCHERI.



# The Dermal Appendages of the Ferns.

BY

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**I**N chapter xi of the first volume of my book on Ferns<sup>1</sup> a brief statement was made on their hairs and scales, as providing material that may be used in comparison, with a view to their phyletic seriation. The statement was necessarily concise, and the few pages devoted to this subject, largely occupied as they were by illustrations, appear to have failed to bring conviction in certain quarters. Since at the moment time is available for the task, I take the opportunity of giving a fuller treatment to a subject that should not be so summarily stated as it was in my chapter xi.

The facts required for the discussion have now been ascertained for most of the leading types of the living Filicales, while there is also a reasonable body of information as to the related fossils. Such data will need to be treated inductively. But at the outset it must be realized that various types of appendages may sometimes be present upon the same individual plant; moreover, where this is so there is some degree of method in their distribution. Speaking generally, the simpler types of dermal appendage, such as linear hairs, are found more especially on the distal regions of the leaves, and the more complex, such as branched hairs, bristles, or scales, are present upon the rhizome and the leaf-bases; but frequently that distinction may not be apparent, and especially so in the more advanced Leptosporangiates. In this essay general assent will be assumed to the view that the Eusporangiate Ferns are relatively primitive, and the Leptosporangiate later and probably derivative types: a conclusion that naturally follows from a detailed comparison of them, while it is in accord with the palaeontological record. It even appears doubtful whether the Leptosporangiate type existed at all in the Palaeozoic Period. Moreover, the leading types of the Mesozoic Floras were such as the Osmundaceae, Schizaeaceae, and Gleicheniaceae: and comparison indicates these as intermediate in character between those two leading divisions of the Class.

The facts will be stated in a rough sequence, starting from relatively primitive types, and proceeding to those believed to be derivative. After such a statement has been made it will be possible for the reader to arrive

<sup>1</sup> Cambridge University Press, 1923.

at a scientifically founded opinion as to the validity of the view stated in chapter xi of 'Ferns', vol. i. But almost all the facts now to be assembled were available when that chapter was written. References will be given to the sources from which the facts have been derived, where also illustrations will frequently be found. But it is not thought necessary here to give illustrations: the references should suffice for those who have access to the literature.

#### BOTRYOPTERIDACEAE.

The so-called 'equisetoid' hairs of the *Botryopteris forensis*, as described by Renault, turn out to be simple uniseriate, transversely septate hairs, rather large and stiff. Their peculiarity arises from the fact that the margins of the septa are thrown into deep corrugations, each appearing as though frilled. The result is that the upward-turned processes of the lower cells interlock with the corresponding downward processes of the upper cells. A similar structure has been found by Mr. Williams in *Dicksonia*, and some other living Ferns with uniseriate hairs. In either case the hairs are merely variants on an essentially simple type.

In *Zygopteris*, however, very striking bristles are found, attached by a narrow base and transversely septate, while each segment may be divided by radial longitudinal cleavages, after the manner known in *Dipteris*. But sometimes in *Zygopteris* the secondary walls may run parallel to one another, and a more or less flattened scale is the result. This was demonstrated to me by the late Dr. Kidston; and other investigators are, I believe, examining the details. Meanwhile we see in the appendages of *Zygopteris* a more elaborate structure than in *Botryopteris*, which may be recognized as running parallel with the larger size and more elaborate structure of its shoot.<sup>1</sup>

#### OPHIOGLOSSACEAE.

The Ophioglossaceae are as a rule glabrous in their mature parts; but there is a variety of *Botrychium virginianum* (designated *b. B. lanuginosum*, Wall), in which the leaf-surface is described as 'slightly hairy'.<sup>2</sup> The apical region of other members of the family may bear hairs, or other appendages, though the adult parts may appear glabrous. Thus in *Ophioglossum palmatum* the stock is surmounted by a dense mat of pale-coloured hairs, through which the young leaves emerge.<sup>3</sup> Campbell describes somewhat similar hairs at the base of the leaf in the same species.<sup>4</sup> On the other hand, he depicts epidermal scales in *Helminthostachys*,<sup>5</sup> and describes

<sup>1</sup> See Renault: Flore Fossile d'Autun et d'Épinac, Part II, p. 33. Bower: Ferns, vol. i, p. 199, Fig. 187. Williams: Ann. Bot., 1925, p. 655.

<sup>2</sup> Syn. Fil., p. 448.

<sup>4</sup> Ferns, Fig. 70.

<sup>3</sup> Bower: Ann. Bot., xxv, Pl. XXII, p. 277.

<sup>5</sup> Sc. Fig. 46, Ferns.

them as growing from the base of the leaf, and from the tissue about the stem-apex; and he shows them in section 'between the enclosed leaf and the surrounding sheath in the lower portion'.<sup>1</sup> It is thus clear that the Ophioglossaceae are not glabrous *ab initio*. It would seem probable that they may have become generally, but not constantly and completely, glabrous in relation to their leathery habit. They may probably have been descended from forms with simple hairs, or possibly from such as already bore flattened scales of the type indicated as existing sometimes in *Zygopteris*.

#### MARATTIACEAE.

In the six genera of this family there is some divergence in the detail of the dermal appendages. In *Angiopteris* and *Marattia*, genera in which the compact sori are seated near to the margins of the leaf-segments, short and simple hairs are found, and no scales. These sori link on readily with the marginal tassels of *Zygopteris* or the marginal synangia of *Corynepteris* respectively. But in *Christensonia* and *Danaea* peltate scales are present, as shown by Campbell.<sup>2</sup> In the latter of these genera the elongated sori cover the whole lower surface of the fertile pinnae, while in the latter the rosette-like sori are dotted over the greatly enlarged leaf-surface. Both of these soral states may be held as derivative from the compact marginal state of *Angiopteris* or *Marattia*; and peltate scales accompany them. In *Protomarattia* and *Archangiopteris* (both of which names are phyletically committal and misleading) the sori, which have respectively fused and separate sporangia, are diffuse and elongated, so as to reach almost to the midrib of the fertile pinna; and elongated scales are borne by both genera. Both of these characters, viz. the flattened scales and elongated sori, may be held as secondary and derivative.<sup>3</sup>

#### OSMUNDACEAE.

This family has a consistent palaeontological history back to Palaeozoic time. In many features the living Osmundaceae show characteristics that are held as primitive: of all living Ferns they are probably the most important links between Palaeozoic types and those characteristic of modern times. This conclusion, which emerges from a general comparison of their structure and development, gives a special interest to their dermal appendages. In them hairs only are present. These are soft, unbranched, and uniseriate, and the distal cells of each are developed as mucilage-secreting

<sup>1</sup> Loc. cit., p. 71, Fig. 49, B.

<sup>2</sup> Ferns, p. 150, Figs. 129, 131, and p. 152, Fig. 136.

<sup>3</sup> See Christ and Giesenhagen: *Flora*, 1899, vol. 86, p. 72, Fig. 7. Also Hayata: *Bot. Gaz.*, 1919, vol. lxvii, Pl. I, Fig. 4. Also Ferns, vol. ii, chap. xx.

cells, which are liable to burst on access to water, setting free the secretion.<sup>1</sup> Thus the whole apical region may be covered by a mucilaginous coat that protects it from drought. For us the special interest lies in the fact that the hairs of this very primitive family are all simple and uniseriate.

#### SCHIZAEACEAE.

A similar interest attaches to the Schizaeaceae, but it is enhanced by the fact that this ancient family is represented by four living genera strangely divergent in habit, though naturally linked by the persistent marginal origin of their 'monangial sori'. As Prantl has shown, the three genera, *Lygodium*, *Schizaea*, and *Anemia* bear only uniseriate hairs, of which the terminal cell may develop as a gland. But in *Mohria* he found that the cell-row widened out, with longitudinal divisions, into a flattened scale, though still bearing the terminal gland as in the other genera.<sup>2</sup> *Mohria* thus stands alone in its family in respect of this feature. But it is also advanced in its general habit, its dictyostelic structure, and in its spore-output: especially is it so as compared with the protostelic *Lygodium*. Though all the features do not necessarily march parallel, such parallelism as does exist is worthy of note, and in the present instance the existence of scales in *Mohria*, as against their absence in the rest of the family, may be held as a feature of advance.

Simple hairs are found also in the related family of the Marsileaceae.

#### GLEICHENIACEAE.

This family presents an extraordinary variety of character in its dermal appendages, so much so that were it not for the great preponderance of evidence from other sources, and from considerations of development, it might appear that argument from them at all would be fallacious, or at least unreliable. Their range in the family extends from soft, simple, uniseriate hairs, to stiff, branched bristles, or flattened scales, seated each upon a massive emergence, and in some forms these different types of appendage may be found in near relation one to another.

In the genus *Gleichenia* the xerophytic section *Eu-Gleichenia* has broad scales protecting the dormant leaf-apices, and also on the leaf-bases and the rhizomes, where they are seated upon more or less prominent emergences. Simple soft hairs are often associated with them. In such a species as *Gl. (Mertensia) flabellata* the scales bear stiff marginal spines, and this state links up with that seen in *Gl. (Eu-Dicranopteris) pectinata*, in which no flattened scale is seen at all, but a tuft of stiff bristles seated upon

<sup>1</sup> Gardiner and Ito.: *Ann. Bot.*, i, p. 27, Pl. IV.

<sup>2</sup> Prantl: *Die Schizaeaceen*, Leipzig, 1881, pp. 35-8, Pl. III, Fig. 36, A.



a massive emergence.<sup>1</sup> Such appearances suggest that the flattened scale is a xerophytic derivative by webbing of stiff, tufted hairs: the converse is possible, though hardly probable. It is worthy of special note that two species of *Gleichenia*, separated on that account under the sectional name of *Eu-Dicranopteris*, have no flattened scales: and that they also stand apart from the rest of the genus in their specially advanced characters both of anatomy and of sorus.<sup>2</sup>

Professor J. McL. Thompson has shown how varied are the dermal appendages in *Stromatopteris*, ranging from simple hairs to irregular and branched scales, or lanceolate scales perched on conical emergences.<sup>3</sup> These all appear generally to conform to the types seen in *Gleichenia*, and they probably present the results of high specialization in relation to the xerophytic and partially underground habit of this peculiar plant. It would, however, be interesting to have the details of them as seen at the apical region and on the surface of the underground rhizome as a whole.<sup>4</sup> On the other hand, that peculiar xerophytic plant *Platyzoma microphyllum*, with its highly advanced type of sporangium and small spore-output, has only simple hairs, long and silky in texture, covering the abbreviated rhizome and its apical region.

Facts so varied as these within what is certainly a very natural and ancient family present unusual difficulties for theoretical interpretation. But such difficulties in a single family should not be held as subversive of conclusions based upon a general comparison of Ferns at large. The leading circumstance affecting them appears to have been the prevalent xerophytic habit of many of these Ferns. This has probably favoured the development of flattened scales even in so primitive a family. But the fact that two species, so distinct from the rest of the genus as are *Gl. (Eu-Dicranopteris) pectinata* and *linearis*, present no scales, has the effect of marking them specially apart from the rest of the genus, and of accentuating the comparisons with other hair-bearing genera, such as *Lophosoria* and *Metaxya*, which show similar characteristics of their anatomy and of their sori.

#### HYMENOPHYLLACEAE AND LOXSOMACEAE.

In these strictly marginal Ferns hairs are present, and with the exception of the peculiar marginal shields on the leaves of *Trichomanes membranaceum* with their thalloid form, scales are absent from the Hymenophyllaceae.

The hairs are frequently simple, but they may be stiffly branched, as they are on the leaves of many species of *Trichomanes*, or they may develop as stiff bristles, as in the rhizomes and leaf-bases of *Loxsomopsis* and

<sup>1</sup> Ferns, vol. i, Fig. 192.

<sup>2</sup> Ann. Bot., xxvi, 1912, p. 269, &c.

<sup>3</sup> Trans. Roy. Soc. Edin., lii, 1917, p. 137, &c.

<sup>4</sup> Compton : Linn. Journ., xlv, 1922, p. 453.

*Loxsonia*. In these the upper part is a simple chain of cells, but the hair widens conically downwards, with numerous cell-divisions both longitudinal and transverse. It is not a flattened scale, but the base of each hair appears as a pear-shaped boss. On the upper regions of the leaf the hairs are soft and curved, without basal swellings.

#### DICKSONIACEAE.

In this family, which includes (*a*) the *Thyrsopterideae*, (*b*) the *Dicksonieae*, and (*c*) the *Dennstaedtiinae*, scales are consistently absent, and the dermal appendages are simple hairs. In *Thyrsopteris* they may appear on the rhizome as stiff brown bristles, but on the upper leaf they are soft and mucilaginous. In the *Dicksonieae*, even in the large dendroid species, hairs only are present. Those on the rhizome of *Cibotium* are specially long and silky, as in *C. Barometz*, the 'vegetable lamb'. The *Dennstaedtiinae* are notoriously hairy, and without scales, for it was this feature that suggested to Kuhn the ground for separating them as the main constituent of his 'Gruppe der Chaetopterides' (Berlin, 1882). Prantl adopted this grouping later, designating them together with *Saccoloma* by the name of the *Dennstaedtiinae*.<sup>1</sup> This persistent retention of hairs in the marginal series is a fact of which too little notice has hitherto been taken.

#### PLAGIOGYRIACEAE, BOWER.

The genus *Plagiogyria* is so isolated and distinct in its characters that it should be given ordinal rank. In the search for its affinities the eye of the systematist has usually been directed towards derivative rather than primitive sources, with the result that it has been ranked as a section of the genus *Lomaria*,<sup>2</sup> while in Christensen's 'Index' it is placed with *Cryptogramme*, *Llavea*, and others under the *Cheilanthes*, as a section of the *Pterideae*. But if attention be directed rather to the more primitive types, there appears good reason to rank it as related in some degree to the *Schizaeaceae*, but more particularly to the *Osmundaceae*, and especially to *Todea*,<sup>3</sup> an affinity which is strengthened both by its anatomy and by the nature of its dermal appendages.

These are exclusively hairs: scales are absent. The hairs themselves are simple, soft, uniseriate, and often curly. They form a dense felt as in the *Osmundaceae*; and, as in them, the distal cells are mucilaginous, so that the whole apical region is protected against drought. So close is this protection over the enlarged leaf-bases and upper leaves of *Plagiogyria* that aeration, which would otherwise be interfered with by the mucilaginous

<sup>1</sup> Abhandl. d. Kön. Bot. Gart. Breslau, I, i.

<sup>2</sup> Syn. Filic.

<sup>3</sup> See Ferns, vol. ii.

coat, is secured by pneumatophores that project through the covering. Though *Plagiogyria* has been ranked with the Cheilanthes in recent classifications, it has been shown that flattened scales are present in *Llavea*, in *Cryptogramme*, and in *Trismeria*,<sup>1</sup> while they are also seen in *Ceratopteris*.<sup>2</sup> Whatever the nearest affinity of the genus may be, speaking from the point of view of general comparison the presence of simple hairs and the absence of scales in *Plagiogyria* are significant facts, but they stand out all the more plainly since scales have been demonstrated in the other genera named above, and its hairs may accordingly be held as indicating a relatively primitive state, a conclusion borne out by other features also.

#### PROTO-CYATHEACEAE, BOWER.

Under this name I have associated two old genera of Presl, viz. *Lophosoria*, Presl, 1848, and *Metaxya*, Presl, 1836, which had been merged by Sir W. Hooker in *Alsophila*. Both are monotypic genera, and both differ from *Alsophila* in having simple sori, with no gradate sequence of the sporangia: both also show a relatively primitive vascular structure. It is, then, significant that in both of these genera hairs are present and no scales, notwithstanding that broad scales are a leading feature of the Cyatheaceae, with which they have usually been ranked. Moreover, looking in the direction of the Gleicheniaceae, which share with the Cyatheaceae certain characters of habit and of anatomy, as well as the superficial position of their sori, it is again significant that the two species now grouped as § *Eu-Dicranopteris*, are also characterized by bearing hairs and no scales. But the stiff hairs of the leaf-base in *Gl. pectinata* are borne upon massive emergences, and similar low emergences may appear at the base of large leaves in *Lophosoria*. All this is in striking contrast with the scaly Cyatheaceae, but it serves to focus attention upon certain species of *Alsophila*, such as *A. pubescens*, Baker, and *A. Taenitis*, Hk., which have habitually been grouped with *Metaxya*, and regarding which the knowledge of the dermal appendages is still deficient. The facts suggest that there has been a transition from the *Eu-Dicranopteris* type to the Cyatheaceous, involving the adoption of an erect dendroid habit, and a gradate sequence of the sporangia: and that parallel with this there has been the adoption of scales in place of simple hairs. The proto-Cyatheaceae appear to occupy a middle position in this sequence of Ferns, all of which share a strictly superficial position of their sori.

<sup>1</sup> Thompson : Trans. Roy. Soc. Edin., lii, No. 14.

<sup>2</sup> Kny. Parkeriaceae, Pl. XXIII, Fig. 8.

## CYATHEACEAE AND CYATHEOID DERIVATIVES.

The presence of profuse scales, often perched upon those massive emergences which provide the characteristic 'armature' of the Cyatheaceae, and their constantly superficial sori, are in strong antithesis to the simple hairs found in the Dicksoniaceae and their constantly marginal sori. Such features with certain others appear to point these two families out as parallel but distinct sequences, which happen, however, to share a prevalent dendroid habit. The relatives and further derivatives of the superficial series are consistently scaly in their dermal protections. This is seen in the whole series of Blechnoid and Dryopteroid Ferns, and accordingly they need not claim our detailed attention here. They share this with the general mass of the modern Leptosporangiate Ferns.

## MATONIACEAE AND DIPTERIDACEAE.

*Matonia* is an ancient type, dating from Mesozoic time, and it preserves many primitive features, among which is a simple superficial sorus. It also bears simple hairs, which are often thin-walled at the base, but they are indurated towards their acute tips. They are similar in general character to those of *Gleichenia pectinata*, but here they are unbranched, and are without any basal emergence. Thus from a comparative point of view they are relatively primitive.

The hairs of *Dipteris* are of essentially the same type. Dense brown bristles cover the axis and the leaf-bases. They widen conically downwards, and many longitudinal divisions appear in the lower cells, but the bristles are not actually flattened, though the insertion may be oval as seen in transverse section. The basal cells are relatively thin-walled, and they show signs of intercalary activity, but in the upper parts the cell-walls are indurated, and each bristle runs out into a stiff terminal spine. This state appears as an advance upon the filamentous hairs of *Matonia*.<sup>1</sup>

## DIPTERID DERIVATIVES.

It appears probable, on general grounds of comparison, that the Dipteridaceae, which were very prevalent Ferns in the Mesozoic Period, have given rise to many types of Ferns living to-day. These may be styled collectively the Dipterid derivatives. Of these one of the most interesting is *Cheiropleuria bicuspis* (Bl.), Presl, which is the sole representative of its genus. It is a native of the Malayan region, and is often associated locally with *Dipteris conjugata*. A detailed examination of it has

<sup>1</sup> Seward : Phil. Trans., vol. cxcl, 1899, p. 171. Seward and Dale : Phil. Trans., vol. cxciv, 1901, p. 487.

shown it to be protostelic, with a very primitive mode of origin of the leaf-trace, reminiscent of that of *Gleichenia*. Its sorus is acrostichoid and 'mixed', while the sporangia show in structure and segmentation interesting features comparable with those of *Dipteris*, and of other primitive Superficiales. With such general features it is then a point of special interest to find that *Cheiropleuria* bears hairs only, and no scales; and that their details compare closely with those of *Matonia*, which we have seen to be simpler than those of *Dipteris*. Thus *Cheiropleuria* may be held as primitive in its dermal appendages and in its stelar structure, though in its Acrostichoid and 'mixed' sorus, and its diplodesmic sporophyll, it is among the most advanced. But against this is to be placed the fact ascertained by Hamshaw Thomas, that its spore-output per sporangium is typically 128—a most unusual exception among Leptosporangiate Ferns, and recalling in the most suggestive way the relatively high spore-output of certain Mesozoic Dipterids.

On the other hand, the sporophylls of *Cheiropleuria* provoke at once a comparison with *Platyserium*, with which it shares the irregularly forked form, the Acrostichoid sorus, and the diplodesmic vascular supply to the fertile region. But the vascular supply of the stem of *Platyserium* appears as a highly advanced result of disintegration of the solenostelic state seen in *Matonia* and *Dipteris*, while the sporangia and the spore-output appear of a type usual for Leptosporangiate Ferns. Again we refer to the dermal appendages for such evidence as they may bring, and we find, in place of the simple hairs of *Cheiropleuria*, that scales are present on the rhizome of *Platyserium*, and stellate hairs protect the young sori. The dermal appendages have advanced together with the other characters.

The leading types of relatively primitive Ferns have now been considered. It will not be necessary to pass in review the great bulk of the Leptosporangiate Ferns that remain, for in one form or another the flattened scale is the prevalent form of dermal appendage in them. The very fact that the presence of hairs and the absence of scales in the Dennstaedtiinae appeared so important to Kuhn was founded upon its unusual character among Leptosporangiate Ferns. We have in fact taken into our view all the most important examples of the occurrence of hairs and not of scales as dermal appendages, and we are therefore in a position to estimate this feature for the purpose of phyletic argument.

Prantl (Schizaeaceae, p. 38) first drew the distinction clearly between hairs (*pili*) and scales (*pili paleacei*), regarding the former as primitive and the latter as derivative from them. Our questions will then be (1) what evidence is there for such a structural advance? (2) is the suggested origin of flattened scales the only one in Ferns? and (3) is it physiologically probable? The evidence that the advance has actually taken place is

primarily structural and developmental. The simple hair, consisting of a single row of cells, is seen in such a natural family as the Schizaeaceae. It is there terminated by a glandular cell: whether the secretion be intracellular or between the cuticle and the inner wall is a difference of detail: sometimes the gland is sessile.<sup>1</sup> In all the genera, except *Mohria*, the hair consists of a single row of cells, but in *Mohria* the cells of the single row are subsequently divided longitudinally. From these facts Prantl drew the natural conclusion that the simple hair is the original type, which in many circles of affinity develops into a flattened form. The details of development of ramenta given by Sadebeck<sup>2</sup> lead to a like conclusion, for he finds that each is referable in origin to a single cell of the still active apical meristem. Tracing it in *Asplenium* he describes how this cell promptly divides, the distal cell forming the tip of the scale, while the rest is of intercalary origin with divisions longitudinal and transverse. It may assume a large surface-area, often becoming peltate at the base, and even several layers in thickness. In some genera, as in *Elaphoglossum*, which is characterized by peculiarly elaborate scales, the stalk when fully formed is never cylindrical, but always flattened, and composed of a plurality of cells ranged side by side. Nevertheless, the generalization of Prantl may be held as applicable even to such scales as these.<sup>3</sup>

The same reasoning applies also to stiff bristles. A good example is seen in *Dipteris*, which differs from its congener *Matonia* in having its rhizome and leaf-bases covered by bristles, that differ from the simple hairs of *Matonia* by having longitudinal divisions in the cells near to the base. Seward has shown how these originate late, so that the ontogeny of *Dipteris* reflects the presumed origin of the bristle from the simple hair.<sup>4</sup> Examples of similar progressions, which might be extended indefinitely, support the generally accepted view that progressions from the simple hair to the stiff bristle or to the flattened scale have happened repeatedly and polyphyletically.

Further, there remains the question whether all flattened scales have originated in this way. It is not here asserted that they have. It is possible that scales may have appeared phyletically as such, and it is open to any one to produce evidence that they did so arise: but hitherto such evidence is wanting.

Corroboration of the conclusion as above stated may be derived from the fact that in many circles of affinity the progression from hairs to scales has marched parallel with advances in other and quite distinct characters, so that the evidence from them all becomes cumulative. A few examples will suffice to make this clear.

<sup>1</sup> Prantl: loc. cit., Figs. 36, A, B, C.

<sup>2</sup> Engler and Prantl, i, 4, p. 60.

<sup>3</sup> Christ: Monographie des genus *Elaphoglossum*, Zurich, 1899.

<sup>4</sup> Phil. Trans., vol. cxclv, p. 487.

(i) On comparative grounds it is held that *Angiopteris* and *Marattia*, the one polysporangiate and the other synangial, represent a relatively primitive type in virtue of their closely intramarginal sori, and they both have hairs only. But in the other four genera, where the sori are either extended along the veins or dotted over an enlarged surface, these being held as features of advance, scales are present.

(ii) In the Schizaeaceae, *Lygodium* is protostelic, and has a relatively large spore-output per sporangium, and it has hairs only; *Mohria*, which is dictyostelic and has a smaller spore-output, is the only genus of the family with scales.

(iii) In *Lophosoria*, where the relatively primitive vascular system is solenostelic or simply dictyostelic with a slightly interrupted leaf-trace, and the sorus is simple and unprotected, hairs only are present; but in *Cyathea* the complicated dictyostele has accessory medullary and sometimes also cortical strands, and a highly divided leaf-trace, while the gradate sorus has an effective basal indusium: and here the appendages are profuse scales.

(iv) In *Plagiogyria*, with its solenostele or primitive dictyostele, and sporangia with oblique annulus, hairs cover the surface; but in *Llavea* and *Trismeria* and others, with vertical annulus and dictyostelic structure, scales are present.

(v) In *Dennstaedtia*, with its solenostelic axis and gradate sorus tending towards a mixed character, hairs are present; in *Davallia*, with its advanced disintegration of the stele and leaf-trace, and with a definitely mixed sorus, scales cover the surface.

(vi) In *Cheiropleuria*, which is protostelic with a very primitive leaf-trace, and an unusually high spore-output per sporangium, the dermal appendages are hairs; but in *Platyccrium*, with its highly disintegrated vascular system and lower spore-output, scales are present.

These examples are cited as facts of experience. They show that though the march of advance is not constantly parallel in respect of all the criteria of comparison, there is a high degree of uniformity, which makes itself evident in the general result, viz. that where hairs only are borne, other features, and often a plurality of them, confirm the relatively primitive character of the Fern in question; other Ferns held to be related may bear scales, in which case they are habitually advanced in other features also. But over and above such specific examples there remain the still more general facts of experience, viz. that the advanced Leptosporangiate Ferns all bear scales, though simple hairs may be intermixed with them; conversely, it is only in relatively primitive forms that scales are actually absent.

Lastly, the question of physiological probability may be raised. *Prima facie* no one can doubt the greater protective efficiency, whether mechanical or physiological, of scales over hairs. An examination of the leaf of *Asplenium*

*Ceterach* or of the rhizome of *Phlebodium aureum* will demonstrate it ; but more particularly a section, transversely through the part that bears them, shows how elaborate may be the overlapping of the scales in Ferns liable to be exposed to drought. The advantage clearly lies with the scale-protected Fern.<sup>1</sup>

For ordinary minds such evidence as that adduced above in support of the general thesis will doubtless be sufficient. There should not be any need to restate here what is so clearly contained in the preface to my first volume on the Ferns ; viz. that the whole argument is inductive, and the conclusions tentative. Among those conclusions is the opinion that, on present showings, the simple hair should be held as primitive, and the scale or bristle derivative from it ; also that the dermal appendages are features fit for use, together with other characters, in phyletic argument. But the conclusions so arrived at will always be open to amendment as new facts or arguments emerge.

<sup>1</sup> Ferns, vol. i, Figs. 188-90.



# The Extraction of Sap from Living Leaves by Means of Compressed Air.

BY

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TO test whether a current flowing through the xylem, though produced artificially, could be used for the transport of carbohydrates, Dixon and Ball (1) enclosed branches of *Sambucus nigra* and *Tilia americana* in a strong steel cylinder, in such a way that the cut end of the stem protruded. Compressed air at pressures up to twenty atmospheres was admitted into the cylinder, and the liquid which exuded from the cut end of the branch (2–3 c.c.) was examined. It was found to be completely, or almost completely, free from reducing sugars. If, however, the leaf-cells were made permeable by means of toluene vapour, the liquid exuded contained as much as 5 per cent. of sugars. Dixon and Ball concluded that no part of the sugar from the leaves could be driven from the tracheae of the supporting branch by pressure, unless there was first of all a change of permeability in the leaf-cells.

In connexion with some experiments on the hydrogen-ion concentration of leaf-cells, we had occasion this summer to employ Dixon and Ball's apparatus to force back the sap from the leaves of *Clerodendron trichotomum*. The quantity of sap exuded (10–20 c.c.) was relatively large, equivalent to nearly half the fluid in the leaf-cell vacuoles. Further, the leaf-cells appeared to suffer no injury, and regained their original water-content if the leaves were left with their petioles in water. By combining the exuded sap from several separate experiments, sufficient was collected to enable a fairly comprehensive micro-analysis to be made. Carbohydrates and organic nitrogenous compounds were present, but only in relatively small amounts.

As there was no apparent change in the permeability of the leaf-cells this observation is interesting, but further research will be needed before any physiological significance can be drawn from it.

<sup>1</sup> A grant from the Department of Scientific and Industrial Research is gratefully acknowledged.

## EXTRACTION OF THE LEAF-SAP.

The shoots were cut about 10 a.m. during the latter half of September 1925 from a tree growing against a wall facing south at the Physic Gardens, Chelsea. They were placed in Dixon and Ball's apparatus within half an hour, and sap forced out by admitting compressed air from a cylinder. In all, fifteen shoots were treated, and about 200 c.c. of clear colourless sap collected. The two experiments described in detail below will bring out the chief points of interest.

*Case 1.* A shoot weighing 57 grm. and bearing seventeen large and three small leaves was enclosed in the apparatus. The air-pressure inside was raised slowly, and at twelve atmospheres drops of liquid began to exude from the protruding end of the stem. The liquid collected resembled pure water: no trace of brown colour—characteristic of the liquid which can be expressed from cytolyzed leaves—was present. Details of the samples collected are given in Table I.

TABLE I.

*Showing Details of the Sap exuded from a Shoot of Clerodendron trichotomum weighing 57 grm.*

<i>Time taken for sample of sap to exude.</i>	<i>Air-pressure.</i>	<i>Volume of sample of sap.</i>
Min.	Atmospheres.	c.c.
3	12-18	1.8
6	18-20	2.7
4	20	2.3
15	22	2.9
41	22.5	4.1
23	22.5	0.8
Total 92		14.6

At the end of the experiment the shoot was removed from the apparatus. The leaves were quite flaccid and weighed 27.5 grm.: after drying in an oven at 108° for twenty-four hours they weighed 9.3 grm. The petioles weighed 6 grm. and after drying 1.5 grm.; the stem 8.5 grm. and after drying 3.75 grm. A comparison of the total solids and water-content of this shoot, after the experiment, with that of a shoot which had not been treated with compressed air is given in Table II; clearly the major part of the exuded sap has been forced back from the leaves.

*Case 2.* The weight of the shoot before placing in the apparatus was 90 grm. The air-pressure was raised fairly rapidly to twenty atmospheres and then kept steady. The successive samples of sap collected were as follows: 2.5 c.c. in 5 min.; 4.5 c.c. in 8 min.; 4.8 c.c. in 15 min.; 5.0 c.c. in 41 min. The total volume of sap exuded was 19.2 c.c.

TABLE II.

*Comparison of a Treated and an Untreated Shoot of Clerodendron trichotomum.*

	Original weight of untreated shoot, 50 grm.		" " treated shoot, 57 grm.	
	<i>Total solids.</i>		<i>Total water.</i>	
	<i>Untreated.</i>	<i>Treated.</i>	<i>Untreated.</i>	<i>Treated.</i>
	grm.	grm.	grm.	grm.
Leaves	8.8	9.3	28.3	18.2
Stem	2.7	3.7	3.5	4.7
Petioles	1.3	1.5	5.4	4.5
Exudate				14.6
Total	12.8	14.5	37.2	42.0

After the experiment the leaves, with petioles attached, weighed 51 grm. and the stem 19 grm. The leaves were soaked in water for about a quarter of an hour, but still remained quite flaccid. They were then placed on wire trays, suspended over water in such a way that the end of the petiole was immersed. After twenty-four hours the leaves regained their normal turgidity, and weighed 68 grm., a gain of 17 grm. At the end of another twenty-four hours the leaves still appeared to be quite turgid, though their weight had fallen to 65 grm. The petioles were then removed and were found to weigh 10 grm.

Now the experiment described in Case 1, and others not quoted, had shown that the stem does not lose any appreciable weight when subjected to the air-pressure. We may conclude, therefore, that in the present experiment the leaves originally weighed 61 grm. Deducting 14 grm. for leaf solids we obtain a water-content of 47 grm. At least 17 grm. of this has been forced out by the air-pressure, an astonishingly high proportion when we consider that the cells appear to have been uninjured, and that a definite amount of water must be held by the cell-wall and cytoplasm.

It may be mentioned that after being subjected to the air-pressure, the stem, but not the petioles, becomes air-locked and will not draw up water. Consequently the flaccid leaves will not recover their normal turgidity unless detached from the stem.

#### ANALYSIS OF THE SAP.

The sap collected from the first few experiments was used to establish the presence of reducing sugars; a quantitative analysis was made with the sap collected from six shoots during the first few days of October. Solution A consisted of the first 6 c.c. exuded from each shoot; solution B the rest of the liquid exuded, varying from 6 to 9 c.c. The methods of analysis used were as follows:

*Total solids and ash.* 5 c.c. were evaporated to dryness and dried at

108° for twenty-four hours. The residue was in each case white and very hygroscopic. This was ashed to constant weight at a low temperature over an Argand burner. The ash was white and non-hygroscopic.

*Reducing sugars.* Hexoses were determined by Maclean's (2) method for 0.2 c.c. of blood. Disaccharides were determined by difference after inversion with HCl. The quantity of sap available was too small for a more extensive analysis of carbohydrates to be made.

*Total nitrogen.* Determined by micro-Kjeldahl, using N/100 standard acid and alkali.

*Free ammonia.* As the solution was colourless the free ammonia was determined by direct Nesslerization, using a Hellige colorimeter. The amount of ammonia in solution A could just be determined; 0.0017 mg. per c.c.: solution B gave no appreciable colour with the reagent.

*Amide nitrogen.* Determined by Sachs's method. A sample was hydrolysed with 4 per cent. HCl for three hours, neutralized, and the free ammonia in the solution determined. The increase due to hydrolysis was taken as a measure of the acid amides (asparagine and glutamine) originally present in the solution. Both solutions give a relatively heavy precipitate with mercuric nitrate, but as the inorganic salts in the solution have not been determined, this cannot be accepted as evidence of the presence of asparagine or glutamine.

*Nitrates.* The brucine test was negative in both cases.

*Protein.* A weak violet biuret reaction is given by solution A, and a very weak reaction by solution B. As lead acetate gives only a faint precipitate after standing twenty-four hours, it is improbable that more than a trace of protein can be present.

Table III gives the results of the analysis of solutions A and B. These results must be interpreted with caution. Substances capable of reducing Fehling's solution are undoubtedly present, and there is undoubtedly an increase of free ammonia after mild hydrolysis; but until we have had an opportunity of examining larger quantities of this sap, the suggestion that sugars and acid amides are present must be treated with reserve.

Solution A contains no inorganic nitrogen, other than a trace of free ammonia. About one-third of the nitrogen would appear to be present as acid amides: the remainder is present, probably, as amino-acids, bases, or more complex substances of a similar nature. The latter groups of substances contain 6–10 per cent. of nitrogen, so they would account for most of the organic solids not represented in the table.

Solution B contains about twice as much organic nitrogen as amide nitrogen, so that acid amides and sugars would appear to account for practically the whole of the organic solids.

These analyses are in striking contrast to that given by Wormall (3) for the sap exuded by root-pressure from the vine. In this case the whole

of the nitrogen was inorganic (nitrate and nitrite), the reducing sugars consisted almost entirely of hexoses, and more than one-half of the organic solids consisted of organic acids (oxalic, tartaric, malic and succinic). The concentration of total solids was about the same as that found in the present experiments—1.56 mg. per c.c., of which 0.56 mg. was ash.

TABLE III.

*Analysis of Sap collected from Six Shoots of Clerodendron trichotomum.*

	<i>Solution A.</i>		<i>Solution B.</i>	
	<i>First six c.c. exuded from each shoot.</i>		<i>Rest of the liquid exuded from each shoot.</i>	
	mg. per c.c.	% total solids.	mg. per c.c.	% total solids.
Total solids . . . . .	2.10		1.10	
Ash (soluble in water) . . . . .	0.84	40.0	0.70	63.6
Ash (insoluble in water) . . . . .	0.10	4.8	0.20	18.2
Organic solids . . . . .	1.16	55.2	0.20	18.2
Hexoses . . . . .	0.08	3.8	0.02	2.3
Disaccharides . . . . .	0.10	4.8	0.05	4.6
Acid amides (calculated as asparagine)	0.12	7.1	0.09	8.3
Total nitrogen . . . . .	0.107		0.021	
Ammonia nitrogen . . . . .	0.0017		0.0	
Amide nitrogen . . . . .	0.017		0.010	

# DISCUSSION OF RESULTS.

The analyses given in Table III show that the sap exuded from the shoots contains inorganic and organic material in solution; also, from the evidence given earlier, there is no doubt that the major part of this sap has been forced out of the leaves.

The question, then, at once arises as to whether the material found dissolved in the sap also came from the leaves, or whether it had been washed out of the vessels of the stem by the water-current forced back from the leaves.

In Case 1, quoted above, the stem was 46 cm. long, was slightly tapered, and had a mean diameter of cross-section of 0.5 cm.; its volume was therefore about 9 c.c. An examination of a section of the stem showed that the area occupied by the wood was not more than one-third, and of the phloem not more than one-thirtieth, of the total area of cross-section. The total volume of the wood was therefore 3 c.c. and the phloem 0.3 c.c., amounting to only one-quarter of the total volume of liquid forced out of the shoot.

The tracheae of the wood were fairly numerous, and there would seem to be no doubt that the major part of the exuded sap has passed down through them. As these tubes are dead tissue the sap passing down would remain unchanged in composition, unless, of course, the living cells in contact with the tracheae gave up sugar, &c., to the flowing sap.

TABLE IV.

*Showing the Sugar-Content of Successive Samples of Sap from a Shoot of Clerodendron trichotomum.*

<i>Time taken for sample to exude.</i>	<i>Pressure.</i>	<i>Volume of sample.</i>	<i>Reducing sugars.</i>		
			<i>Hexoses.</i>	<i>Disaccharides.</i>	<i>Total.</i>
Min.	Atmospheres.	c.c.	mg. per c.c.	mg. per c.c.	mg. per c.c.
3.5	0-20	2.4	0.120	0.155	0.275
11	20	4.3	0.020	0.070	0.090
45	20	5.0	0.010	0.070	0.080

The remainder of the sap may have been forced down through the sieve-tubes of the phloem; in which case the contents of the latter would probably be forced out ahead of the sap and enrich the first few c.c. exuded. The analysis given in Table IV shows that the first two c.c. contain a much higher concentration of sugars than the remainder of the exudate, which appears to confirm the supposition that part, at least, of the sap is descending through the sieve-tubes. It will also be observed that after the initial high concentration the sugar-content falls to a steady value, as though this sugar were an integral part of the sap forced out of the leaf, and not a product washed out of the stem vessels. If this is so, then solution A represents the sap as forced from the leaf, and solution B represents similar sap enriched with the contents of the sieve-tubes.

TABLE V.

*Analysis of the Vacuole Fluid of the Leaf-cells of Clerodendron trichotomum.*

	<i>Mg. per c.c.</i>	<i>Percentage of total solids.</i>
Total solids . . .	92.2	
„ ash . . .	41.1	46.5
„ organic solids . .	51.1	53.5
„ hexoses . . .	13.1	14.1
„ disaccharides . .	4.1	4.4
„ nitrogen . . .	1.4	1.5

TABLE VI.

*Comparison of the Total Solids in the Exuded Sap and in the Vacuole Fluid of the Leaves. (Case 2, p. 492.)*

	<i>Solids of vacuole fluid.</i>	<i>Solids of exuded sap.</i>	<i>Ratio.</i>
	mg.	mg.	%
Total solids . . .	4,140	22.0	0.5
„ ash . . .	1,850	18.0	1.0
„ organic solids . .	2,290	4.0	0.2
„ hexoses . . .	589	0.4	0.1
„ disaccharides . .	180	1.0	0.6
„ nitrogen . . .	61	1.1	1.8

It is of interest to compare this sap, forced out of the leaves by air-pressure, with the solids originally present in the vacuole fluid of the leaf-cells. The leaves contain 76 per cent. of water; for the present purpose it is assumed that 80 per cent. of this belongs to the vacuoles. Table V gives the analysis of the vacuole fluid. Assuming that the sap forced back from the leaves has the composition of solution B, the total solids exuded in the experiment described under Case 2, p. 492, are given in Table VI, together with the solids present in the leaf-cell vacuoles. It will be seen that only 0.5 per cent. of the total vacuole solids have been forced out of the leaf, as compared with nearly 50 per cent. of the vacuole water.

The expenses of this research were defrayed by a grant from the Royal Society.

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# Mesembrioxylon rhaeticum, a Triassic Conifer.

BY

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With six Figures in the Text.

A SPECIMEN of coniferous wood came into my hands some time ago, collected by the late Mr. John Storrie, of Cardiff, from the Penarth Beds, of Rhaetic age, which merits notice both on account of the low horizon from which it comes and for certain peculiarities of its own.

There can be no doubt as to its age, for the Penarth Beds are classic ground to geologists, and Mr. Storrie was thoroughly familiar with the neighbourhood. Viewed in the light of this antiquity, even the imperfect features of the present material become of interest.

The specimen consists of a single radial section of secondary xylem. No other material belonging to the same type has been discovered, either in bulk or in section; but fortunately it is the secondary wood which yields just those points of structure on which comparative investigators have focused attention.

## DESCRIPTION.

The tracheides are not long. The total length of the section is only 7.5 mm. but a considerable number of tracheide ends can be found, and the average length appears to be about 1 mm.

There are no annual rings. The average width of the tracheides is 20  $\mu$  and there is no regular variation of width in measuring in series across the section. This uniformity of growth is in harmony with a Triassic attribution.

There are no resin canals and no xylem parenchyma, which is again suggestive of early date.

The tracheides are sparsely pitted, bearing short, uniseriate rows of up to a dozen bordered pits, with large round pores. The pits are usually separate, only occasionally contiguous, and never flattened. Bars of Sanio

are clearly present in some places at least. In other places they equally clearly are not present, so that this feature is inconstant. The rows of pits are mostly in the neighbourhood of medullary rays, and there are long stretches of imperforate tracheide wall. In one or two places pits appear in alternating series (Fig. 2), an Araucarioid character, upon which, however, too much stress cannot be laid in view of the presence of Sanio's rims (2). Pitting of the tangential walls is to be seen in a few places, especially near the ends of tracheides, which shows an early departure from the ancient Cordaitean type (Figs. 4, 5, 6).

In two places *terminal pits* have also been seen (Figs. as above), apparently connecting end-to-end tracheides with transverse partition walls. All the tracheides show the well-known marking in steep spirals, brought out by the decay of the lignin and not to be confounded with true spiral banding. The latter is, however, regularly found on the radial walls of tracheides, opposite to rows of pits, and it is confined to such regions. Whether this appearance also is to be attributed to *post-mortem* changes cannot be decided, but the preservation appears to be satisfactory in other particulars and so may perhaps be trusted in this.

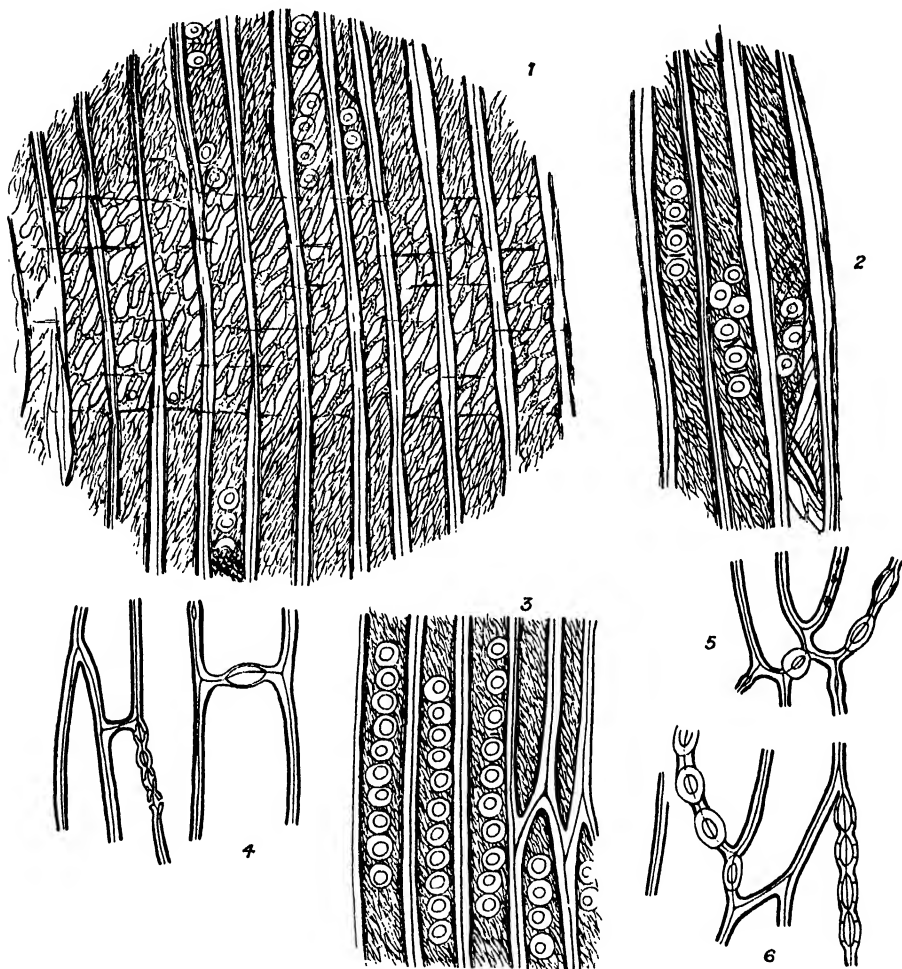
The medullary rays are uniseriate and of no great depth, twelve cells being the greatest depth observed in this section. They are uniformly parenchymatous, and what can be seen of the badly-preserved horizontal walls is smooth and unpitted.

The most peculiar feature of the wood is the pitting of the ray-fields. There are, on the flanks of the medullary rays, series of broad, irregular, spiral bands, continuous above and below with the spiral markings of the tracheides, and forming by their anastomoses a number of obliquely elongated simple pits, the average number of which, per ray-field, is apparently between five and six.

In these latter particulars this wood seems to be unique, but the syndrome of characters certainly falls within the limits of the genus *Mesembrioxylon*, established by Seward (4) to cover the inconstant characters of Gothan's *Podocarpoxylon* and *Phyllocladoxylon*. Seward very justly points out the unreliability of many of the characters upon which the generic separation of fossil gymnospermous wood depends, and the great difficulty of drawing precise lines of separation between the described genera. While fully recognizing this, we find at the same time that the characters of the present wood do agree with those laid down for *Mesembrioxylon*, to which, therefore, it is necessary to attribute it, although it does not agree exactly either with *Podocarpoxylon* or *Phyllocladoxylon*, but partakes of characters of both.

From the latter genus it is distinguished by the numerous, oblique pits in the ray-fields and the presence of Sanio's rims. As against *Podocarpoxylon* there is the absence of xylem parenchyma and the simple

pitting in the medullary rays. From *Cupressinoxylon* it is separated by the absence of xylem parenchyma; from *Cedroxylon* by the unpitted walls of



FIGS. 1-6. 1. Radial view of the medullary ray, showing the unpitted ray-walls with oblique, simple pits in the ray-fields and spiral banding opposite the uniseriate, tracheidal bordered pits. 2. Uniseriate and alternating bordered pits with rims of Sanio. 3. Typical uniseriate tracheidal pitting. 4, 5, and 6. Pits on the terminal and tangential walls of tracheides. All figures magnified approximately 350 diameters.

the ray parenchyma, and from *Paracedroxylon* by the occurrence of Sanio's rims.

*Cedroxylon* appears to be clearly distinguished from *Cupressinoxylon* in respect of its pitted tangential walls in the rays as well as in the less positive character of the absence of xylem parenchyma, while if *Cedroxylon*

*blevillense*, Lignier, be removed to *Paracedroxylon* (which is permitted by the description of the latter genus and appears to be rendered necessary by a close consideration of the original description of *Cedroxylon* by Kraus (3)); this also will have a positive character, depending on the concurrence of unpitted walls in the ray-cells with numerous oblique pores in the ray-field and no rims of Sanio.

Among recent plants it is noteworthy that in *Ginkgo* there is a closely similar arrangement of numerous oblique pits in the ray-fields, conjoined, however, with a different type of tracheidal pitting.

It is in *Xenoxylon* that our present species finds its closest comparison, but that genus is undoubtedly more Araucarian in its tracheidal pitting and in the absence of Sanio's rims. It is interesting, however, that *Xenoxylon* alone, among woods of non-Cordaitean character, reaches down (in *X. conchylianum*, Fliche) to the same Triassic level. Records of *Cedroxylon* go below those of most other Mesozoic conifers, but the only other Rhaetic record for plants of this affinity is *C. pertinax*, Goepp., and that is itself referred to the Jurassic by Gothan (1), while other evidences of greater or as great an age for woods of Abietineous affinity rest also on unsecure records. The present specimen gives us therefore a valuable early link with the immediate derivatives of the Cordaitean stock.

The specimen is in the collection of University College, Cardiff.

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## NOTES.

**REPRODUCTION IN MACROCYSTIS PYRIFERA, Ag.**—Our knowledge of the reproductive processes in the genus *Macrocystis* has hitherto been limited to the descriptions of the sporangia occurring in sori on more or less specialized fertile fronds. These accounts deal for the most part with material from subantarctic regions (Skottsberg, 1907, 1921)<sup>1</sup> or from the west and north-west coasts of America (Setchell and Gardner, 1903;<sup>2</sup> Hoffmann, 1911).<sup>3</sup> It is natural to expect that *Macrocystis* will, however, fall into line with *Saccorhiza* and other Laminariaceae in which the occurrence of a minute gametophyte generation has been established owing to the remarkable discoveries by Sauvageau in 1915 and by others in subsequent years. Evidence has been obtained by the authors that this is almost certainly the case, but has been withheld from publication in the hope of obtaining a more complete series of observations. Unforeseen circumstances have delayed this indefinitely, and it seems, therefore, better to publish such results as have been secured.

*Macrocystis* occurs at the Cape in comparatively shallow water just below the lowest tidal limits and usually protected from the full force of the waves by a belt of *Ecklonia buccinalis*. It is abundant on the rocky shores at Cape Point, Kommetje, Camps Bay, and Sea Point. It is frequently cast up on the shores of Table Bay, which perhaps accounts for the records of its occurrence there by Barton (1896)<sup>4</sup> and by Tyson. The habit is that of the prostrate rhizome-bearing specimens described by Hoffmann (1911)<sup>5</sup> bearing branched hapterons at intervals. The assimilating 'leaves' are borne on stipes of 12–15 ft. in length; the fertile leaves occur on much shorter stipes or are nearly sessile on the rhizome-like horizontal stipes. In our experience, the mature fertile leaves were usually not more than one or two feet in length.

The sori appear mainly on these basal shoots as irregular deep brown patches on both sides of the smooth surface. In three cases out of a considerable number examined, linear sori have also been detected, occupying some of the grooves in the wrinkled assimilating fronds near the base of the stipe. Setchell and Gardner (1903)<sup>6</sup>

<sup>1</sup> Skottsberg, C., 1907: Zur Kenntnis der subantarktischen und antarktischen Meeresalgen, i. Phaeophyceen, Stockholm. Id., 1921: Botanische Ergebnisse der schwedischen Expedition nach Patagonien und dem Feuerlande, 1907–9, viii, Stockholm.

<sup>2</sup> Setchell, W. A., and Gardner, N. L., 1903: Univ. of California Publications, I, Botany.

<sup>3</sup> Hoffmann, E. J., 1911: Ibid., IV, Botany.

<sup>4</sup> Barton, E. S., 1896: Journ. of Bot., xxxi–xxxv.

<sup>5</sup> Hoffmann, E. J., 1911: Loc. cit.

<sup>6</sup> Setchell, W. A., and Gardner, N. L., 1903: Loc. cit.

record a similar position of the sorus in certain specimens from Peru, but do not state whether they regard this as exceptional.

The fertile fronds begin to appear in the spring months (October, November) and continue fertile until the following April at least. In December 1920 material collected by one of us (E. M. D.) from Kommetje was brought to the laboratory and examined. The sea-water in which the material had been placed was full of zoospores, which were sluggish in movement on Dec. 14 and passive and surrounded each with a cell-wall on Dec. 16. On Dec. 31 the spores had apparently made short filaments of two or three cells, but owing to lack of time no drawings were made, and the culture was subsequently thrown away.

The following year, in the autumn (April 1921), a further attempt was made by one of us (M. L.) to obtain a culture of the germinating zoospores. A small por-

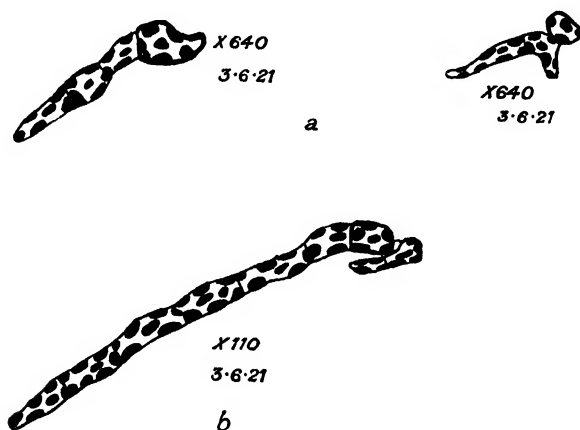


FIG. 1. *Macrocytis pyrifera*, Ag. Camera lucida drawing (M. L.) of germinated zoospores in culture 6-7 weeks old. *a*, presumably male gametophytes,  $\times 640$ ; *b*, presumably female gametophyte,  $\times 110$ .

tion of a fertile frond was cut from a plant of *Macrocytis* growing at Camps Bay, placed in a collecting tube with a small quantity of sea-water and brought into the laboratory (April 15). About two hours after collecting, the material was placed in a covered glass dish containing about 100 c.c. of sea-water and a few cover-glasses. Next day the piece of lamina was removed. Examination of the sea-water in the dish revealed a number of motile zoospores, apparently of two sizes.

On April 26 no sign of germination could be detected in the cultures, but on June 1 filaments of two different dimensions could be clearly detected (Figs. 1, *a* and *b*).

The cultures were left with occasional changes of sea-water until the following spring (October 1921). On Oct. 21 young sporophytes were seen in the stages illustrated in Fig. 2. The younger and smaller sporophytes were in every case attached to a large thick-walled empty cell, resembling closely the stages figured by Sauvageau for the gametophytes of *Laminaria flexicaulis* (1918, Fig. 50, A-M) where the

embryo is seen resting upon the empty thick-walled oogonium. In Fig. 2 an older stage is seen, the embryo having produced a number of colourless rhizoids.

Comparison of Figs. 1 and 2 with the corresponding figures given by Sauvageau (1918)<sup>1</sup> for other Laminariaceae leaves little room for doubt that we have here also a gametophytic generation bearing the embryos. A further point of interest lies in the different behaviour of the zoospores in spring and autumn. In December (spring) the young gametophyte had apparently formed within seventeen days, whereas in April seven weeks were required, and the young sporophytes were

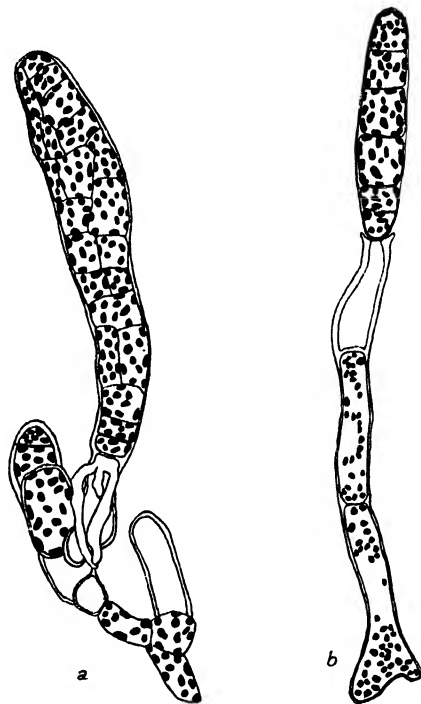


FIG. 2. *Macrocystis pyrifera*, Ag. Camera lucida drawing (M. L.) of young sporophytes from germinated zoospores in culture 22 weeks old.  $\times 110$ .

not detected until nearly five months later, when the winter was over and the spring had arrived again. A similar seasonal effect was noted by Sauvageau for *Saccorhiza*, the fertile gametophytes requiring 2-3 weeks to develop in spring (March, April) and three months or more when started in November, the sporophytes appearing later still, in March. No antheridia were seen in the cultures, but the observations were made under difficulties and were not exhaustive. The empty thick-walled cells at the base of the young sporophyte (Fig. 2) seem to indicate a bisexual gametophyte as in other Laminariaceae.

The tentative observation that the living zoospores are of two sizes is of considerable interest. Most of the Laminariaceae appear to be homosporous,

<sup>1</sup> Sauvageau, C., 1918: Mémoires de l'Acad. des Sciences de France, t. 56, s. 2.

but Sauvageau (1918) noted inequality in size of the zoospores of *Laminaria Cloustoni* and *Saccorhiza bulbosa*, the sex of the zoospore being evidently determined in the sporangium in these cases.

E. M. DELF.

M. LEVYN (née M. MICHELL).

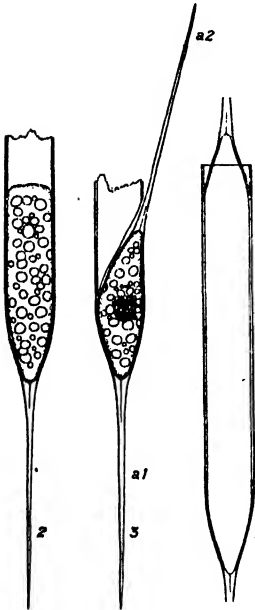
**CELLULAR REGENERATION IN RHIZOSOLENIA SETIGERA, BRIGHT-**

**WELL.**—The marine genus *Rhizosolenia*, (Ehren.) Brightw. (Diatomaceae Centricae, Solenoideae), has delicate, cylindrical frustules, which appear to be very fragile, as broken specimens are common in gatherings. An interesting case of regeneration of cellular form was noticed in a gathering made in tidal waters off the Gower coast, South Wales, in April 1925.

The illustrations will make the matter clear. Fig. 1 shows the normal appearance after cell-division, with the junior, growing semi-cell protruding from the open end of the senior semi-cell.

Fig. 2 shows a broken frustule which has not regenerated. The plasma has rounded off and is filled with oil-drops. In this case no nucleus was included.

Fig. 3 shows another broken frustule, but in this case the nucleus remains, and there has been an attempt to regenerate the complete form. Notice, however, that the newly-formed apex (*a2*) is aligned with the oblique median axis of the upper part of the plasmatic mass, and, consequently, it has broken through and pushed aside the remaining fragmentary stump of the old wall. The nucleus is seen in the centre, surrounded by small oil-drops.



FIGS. 1-3. Cellular Regeneration in *Rhizosolenia setigera*.

R. C. McLEAN.



13

# On the Effect of Light and other Conditions upon the Rate of Water-loss from the Mesophyll.

BY

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With eight Figures in the Text.

A SURVEY of the literature of transpiration reveals the fact that there has been very little study of the part played by the mesophyll in the activities of the leaf other than in photosynthetic assimilation. A multitude of facts and figures are at hand in connexion with transpiration from the leaf as a whole, but few attempts have been made to observe the direct effect of changing external conditions on the mesophyll alone, though it is, of course, from these cells that water-loss mainly takes place.

One of the reasons for the paucity of our knowledge of the behaviour of the mesophyll is that the action of this tissue has not, in most investigations, been separated from that of the stomata. The presence of the stomata imposes a variable factor, the action of which may mask the response of the mesophyll itself to external conditions.

Knight (7) has shown that for small ranges of temperature and humidity the stomatal aperture remains nearly constant, but over larger ranges stomatal movements certainly occur; light also greatly affects the condition of the stomata. It will be clear, therefore, that the action of external conditions on the mesophyll itself can hardly be studied satisfactorily in the intact leaf. It is evident also that little progress is possible without some means of annulling the control of water-loss by the stomata. Up to the present, two methods of effecting this have been proposed. Leaves in which the mesophyll has been exposed by stripping off the epidermis might be used, as suggested by Knight (5). This method, however, is very drastic, and there is the danger of the drying out of the mesophyll cells except in atmospheres of high humidity.

The other method was devised and used by Darwin in 1914 (3, 4). The essentials of the method are, first, to exclude the action of the stomata by blocking them with some substance such as vaseline, and then to put the intercellular spaces of the leaf into direct communication with the outside air by means of slits or incisions in the leaf tissue. Such slits were made with a scalpel usually between the principal veins of the leaf so as to interfere as little as possible with the water-supply. Using this method, Darwin attempted to determine the effect of light and humidity changes on the rate of water-loss from the mesophyll. He came to the conclusion that light increased the transpiration by amounts varying between 10 per cent. and 100 per cent. of that in the dark.

An increase of such an order is certainly remarkably high; to bring about a doubling of the evaporation rate from a moist surface in air of constant evaporating power would require an increase in temperature of the order of  $10^{\circ}\text{C}$ . Darwin's data obtained by the slit-leaf method were therefore closely scrutinized, and two possible sources of error disclosed themselves. The observations were usually begun immediately after smearing and slitting the leaves, thus making no allowance for possible shock- or wound-response; and secondly, the conditions were apparently uncontrolled and largely unknown, the plants in some cases being removed from the window of the laboratory into a dark room, seemingly without accurate records of temperature and humidity being taken.

It seemed therefore advisable to repeat and extend these experiments under controlled conditions, and a special apparatus was accordingly devised for the work. The construction of the apparatus, which is described below, was such as to enable temperature, light, and humidity to be regulated at will, while transpiration was automatically recorded by an electrically operated balance requiring no attention during the experiment. At the same time the temperature of the leaf itself could quickly be determined to  $0.1^{\circ}\text{C}$ . by means of a pair of thermo-couples, one embedded in the tissue of the leaf, the other immersed in ice and water at  $0^{\circ}\text{C}$ .

A brief description of the apparatus is as follows: The component parts comprise (a) the automatic weighing mechanism, (b) provision for continuous air renewal, (c) heating arrangements, (d) humidity regulator, (e) arrangements for determining leaf temperature, and (f) light-supply.

(a) *The automatic balance.* This, like the Blackman and Paine transpiration balance (2), records the loss of a given amount of water by an electric contact made by the rising pan of the balance. In this case, however, the contact liberates a solid compensating weight, at the same time making a mark on a time-chart. The balance is an ordinary laboratory one, turning with one milligram, this type being found to be sufficiently sensitive for all ordinary transpiration work, and more suited for handling than delicate analytical balances. To the left side of the beam is attached

a small arm which can be moved along the beam to any desired position, and from this arm is suspended a small glass bucket by an ordinary cotton thread. Into this bucket are delivered the steel balls from a delivery apparatus, and the effective weight of each ball may be varied by moving the arm and the glass bucket to suitable positions on the beam. The delivery apparatus consists of a suitably bent glass tube leading to the bucket from the exterior of the balance case, and a small electrically operated ball-dropping mechanism delivering balls into the tube.

Upon the left pan of the balance rests a small ebonite cup containing a globule of mercury connected electrically to a terminal on the baseboard of the balance by a fine piece of platinum wire, thin and pliable enough not to damp the swing of the balance yet thick enough not to fuse with the current required to operate the ball-dropper; platinum wire No. 47 S.W.G. was found to suit the purpose admirably. Soldered to another terminal on the baseboard is a piece of stouter platinum wire (No. 18 S.W.G.) burnished to a fine point. This overhangs the globule of mercury in the cup on the balance pan, so that as the specimen under observation loses weight and the pan rises contact is made between the wire point and the mercury. The completion of the electric circuit causes the ball-dropper to deliver a ball into the bucket on the beam, and the pen on the time-drum is pulled over.

The impact of the ball causes the pan to descend, breaks the circuit, and resets the ball-dropper and the pen. The balls used for weights are standard  $\frac{1}{8}$ -in. steel balls for bearings, which are constant in size and weight, and the effective weight of a ball for any position of the bucket on the beam does not vary by more than 0.3 per cent. If, when very small amounts are being recorded, the balance tends to swing back and make a second contact, a small dash-pot containing heavy paraffin oil may be used with success. Since the air in the balance case is constantly being renewed, any mercury vapour rising from the mercury globule on the balance pan is carried away.

(b) *Air-supply.* The balance stands in a glass-sided case, rather like a large balance case, with a sliding front, of a convenient size to allow the balance, plant, and heat and humidity regulators ample room in its interior. The actual case used measures 21 in.  $\times$  18 in.  $\times$  10 in.

The air-supply is provided by a pump of sufficient power to maintain the atmosphere within the case at the required humidity when the plant is transpiring. A small 'Rotoplunge' pump with a  $\frac{1}{8}$  h.p. motor fulfils this requirement. Arrangements are made to admit air at any part of the case desired. The outgoing air is not re-circulated, but is allowed to escape.

(c) *Temperature regulation.* The temperature of the air within the case is regulated by a bimetallic thermo-regulator, conveniently made by soldering a length of thin zinc to a similar length of copper, and bending

the resulting bimetallic strip into an undulating shape. The strip is attached at one end to a pillar, and at the other to an amplifying lever of sufficient length to give a travel of about 1 mm. per degree centigrade, this lever being fitted with an adjustable platinum point which makes contact in a small mercury cup. The bimetallic strip is fixed with the zinc side uppermost, so that as the temperature rises, the platinum point falls and makes contact with the mercury, permitting a low-voltage current to flow through a relay which breaks the high-voltage current through the heater till the temperature again falls. The heater itself consists of a network of No. 30 S.W.G. 'Eureka' wire having resistance enough to give a temperature rise of about  $10^{\circ}$  to  $15^{\circ}$  C. This network is placed beneath a perforated zinc sheet fixed about 1 in. from the floor of the case, and insulated from it by silica board. The incoming air is blown over the heater before reaching the thermostat and ultimately the plant.

(d) *Humidity regulation.* The regulation of humidity is always a difficult matter in transpiration experiments, since the transpiration itself tends constantly to increase the moisture content of the surrounding air. In this case, however, a method was devised which has given excellent results. The humidity of the air in the case is controlled by a bundle of human hair attached at one end to a crank on a shaft, bearing a lever which amplifies changes in the length of the bundle of hair. The hair, which has been rendered free from grease by treatment with sodium carbonate solution followed by ether, expands as the humidity increases. An adjustable platinum point at the end of the lever makes contact in a small mercury cup. This humidity regulator is in series with a relay which 'cuts out' when the humidity rises too high. The high-voltage side of the relay is in series with a 15 c.p. carbon filament lamp. This lamp is held in an ordinary holder, and the whole made waterproof in a suitable manner, e.g. by covering the holder and the base of the lamp with a rubber covering. The lamp and holder are suspended in a large bottle containing a quantity of saturated solution of calcium chloride, the flexible connexion passing through the bung. Two other holes in the bung permit of air being bubbled through the calcium chloride solution. The air from the pump, before reaching the balance case, is bubbled through this bottle. The calcium chloride removes its moisture, dry air is delivered to the case, and the humidity falls. The hair in the regulator shrinks, and the platinum point is raised, breaking the low-voltage relay circuit, and thereby switching on the electric lamp in the bottle. As the lamp heats the solution, the vapour tension rises, and the air entering the case becomes moister, until the humidity within is again high enough to expand the hair sufficiently to make the relay circuit, and switch out the heating lamp, when the humidity of the incoming air will begin to fall again. A precaution which should be observed is that not too much calcium chloride solution be used, or a lag will begin to show, due to

the too slow warming of the solution. Using an adjustable platinum contact, the humidity can be set to any desired value, and should not vary by more than 1 per cent. It may be noted that sulphuric acid was first tried as a drying agent, but the air passing through it apparently carried fine droplets of acid, for it proved lethal to the plants.

(e) *Leaf temperatures.* These were taken by a pair of thermo-couples, one of which was immersed in ice and water in a Dewar flask, the other being embedded in the tissue of the leaf. Copper-‘Eureka’ thermo-couples coated with shellac were used with a low resistance galvanometer, this combination giving readings within  $0.1^{\circ}\text{C}$ . The spear-headed type of thermo-couple was used for the leaf. Fuller details of thermo-couples for leaf work may be found in a paper by Richards (8).

(f) *Light-supply.* Artificial light should always be used in cases where accurate observations are to be made. Here, particularly, the variable amount of heat radiated through the glass sides of the case from surrounding objects exposed to varying illumination during the day is sufficient to change not only the air temperature but even the leaf temperature, so that artificial light becomes a necessity.

A 100-watt gas-filled lamp suspended three feet above the case gives sufficient light to affect the stomata of a leaf as rapidly as bright diffuse daylight, and if, when darkness is required, a cover of thin, black photographic paper be placed over the case, instead of turning out the light, the heating power of the lamp still comes into play. In this way the drop in temperature inside the case need not exceed  $0.2^{\circ}$  or  $0.3^{\circ}\text{C}$ .

The current for working the automatic balance and the marking-pen is supplied from a 4-volt accumulator. The whole of the remaining energy is taken from the mains, a 200-volt direct-current supply, the pressure being cut down to 6 or 8 volts for the relay circuits by an arrangement of resistances.

## EXPERIMENTAL WORK.

The plants selected for treatment were Ivy (*Hedera helix*), *Eupatorium adenophorum*, and *Aster* sp., the first of these being the subject of most of Darwin’s work. The leaves and stems were carefully smeared with vaseline, any excess being removed with absorbent cotton. Incisions were then made between the principal veins, the number of slits required to give transpiration of the normal order being known from previous determinations. This operation usually occupied a period of ten or fifteen minutes. The plants were then put into the constant temperature chamber, the thermo-couple inserted into the midrib or mesophyll, and observations begun.

*I. Effect of Continuous Light on Transpiration from the Mesophyll.*

Two typical experiments of a series of about twenty performed on Ivy are given in detail below. In each case the branch (removed from the plant at the time stated in each experiment) had a new cut surface exposed at the end of the stem. The cut end was then attached to a potometer, usually of the form of a graduated pipette.

TABLE I.

*Experiment B 6.* June 14, 1923. Ivy branch, having four large leaves of the current year. Humidity of air, 70 per cent. Air and leaf temperatures taken about four times an hour. Light, 200 c.p., 3 ft. above the shoot. Leaves vaselined and slit, 11.40 a.m.

<i>Time.</i> p.m.	<i>Water-loss.</i> mg.	<i>Conditions.</i>
12 noon		Observations begun.
12.30-1.0	32.0	Light.
1.0-1.30	31.5	
1.30-2.0	30.5	For temperatures see graph.
2.0-2.30	27.5	Dark.
2.30-3.0	28.5	
3.0-3.30	28.5	
3.30-4.0	27.5	
4.0-4.30	28.0	
4.40		Light.
4.30-5.0	29.0	
5.0-5.30	29.0	
5.30-6.0	30.0	
6.0-6.30	28.5	
6.30-7.0	28.0	Dark.
7.0-7.30	27.0	

In Fig. 1 these results are shown graphically.

TABLE II.

*Experiment B 7.* June 18, 1923. Ivy branch of current year with seven medium-sized leaves. Vaselined and slit, 11.45 a.m.

<i>Time.</i> p.m.	<i>Water-loss.</i> mg.	<i>Conditions.</i>
12.15-12.45	80	Dark.
12.45-1.15	84	
1.15-1.45	94	For temperatures see graph.
1.45-2.15	91	
2.15-2.45	90	
2.45-3.15	85	
3.15-3.45	86	
3.45-4.15	81	
4.15-4.45	84	
4.45-5.15	86	Light.
5.15-5.45	87	
5.45-6.15	87	
6.15-6.45	86	

In Fig. 2 these results are shown graphically.

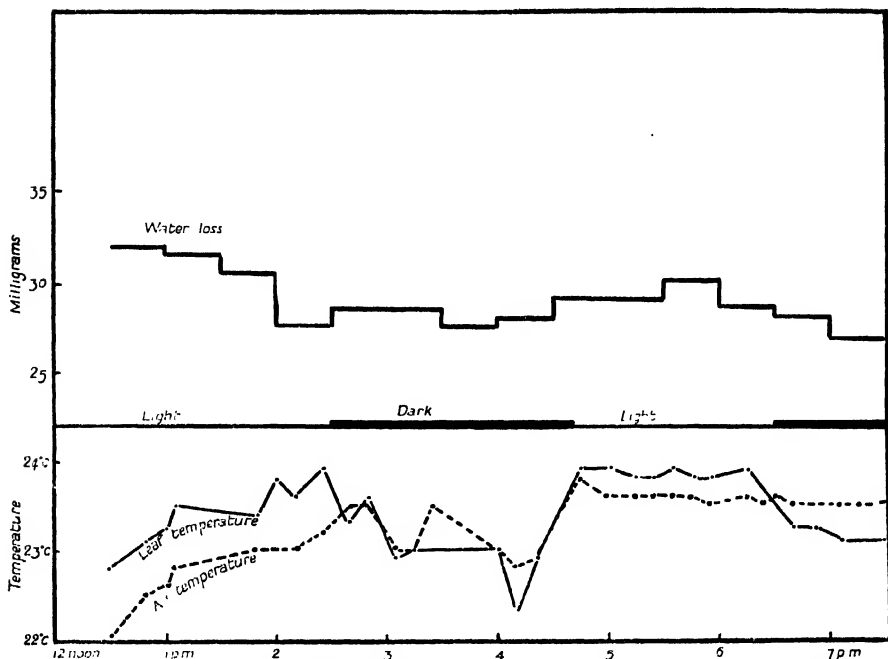


FIG. 1. Expt. B 6. June 14, 1923. Effect of continuous light on the transpiration of Ivy leaves, vaselined and slit 11.40 a.m.

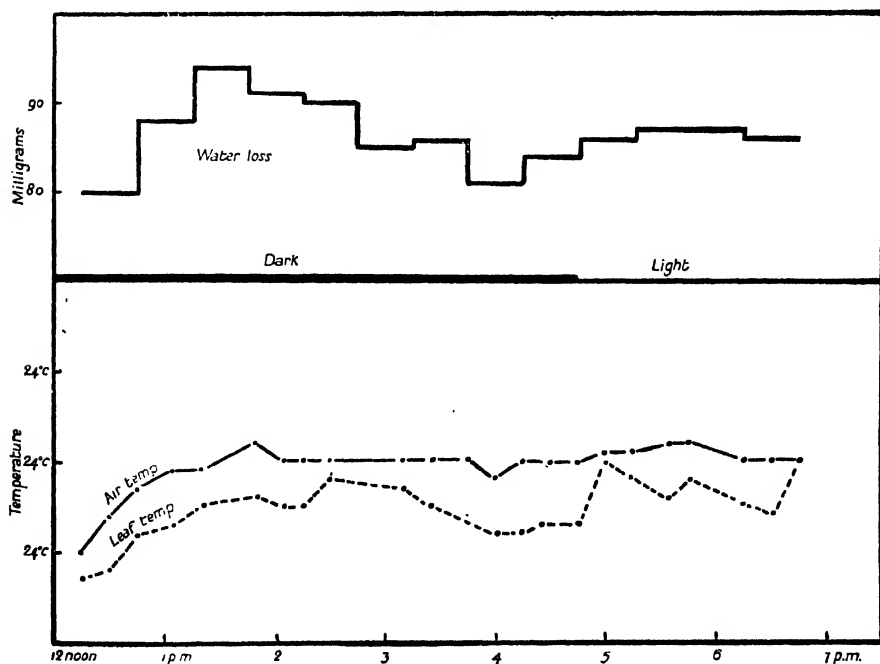


FIG. 2. Expt. B 7. June 18, 1923. Effect of continuous light on the transpiration of Ivy leaves,

Examination of the first of the two graphs given above shows a high rate of transpiration immediately succeeding the operation of vaselining and slitting the leaves; this occurred in many of the experiments performed. In the other experiment the transpiration rises to a maximum after about an hour and a half. This rise to a maximum and subsequent settling down to a constant rate of water-loss after some time characterized about half of the experiments, while the other half showed the phenomenon which appears in the first experiment. Why the maximum rate should occur sometimes at once after slitting, and sometimes only after a period of an hour or so, is not clear.<sup>1</sup> It was during this preliminary, uncertain period, however, that most of Darwin's observations were made, and, since all his experiments were begun in the light, and the plants removed to the dark room when a maximum had been reached, it is not surprising that his results show a drop in the transpiration rate in darkness. It is significant, also, that in experiments where the plants were brought back into the light, in no case was the second 'light figure' as high as the first. The series of graphs, of which the two shown above are examples, demonstrate that, for the period immediately following the preparation of the leaves, the shape of the curve is the same whether the experiment is begun in light or darkness. Thus, had Darwin started his experiments in the dark, his results would in all probability have been reversed.

The irregularity in the transpiration rate just described is only a passing phenomenon, for after a few hours the rate steadies and remains relatively constant for one or two days, when wilting supervenes. It was only during this period of constant rate of water-loss under the constant conditions that the observations on the effect of light were carried out. The shoots under examination were exposed to light and darkness, or vice versa, for varying periods, and in some cases intermittent periods of one or two hours' light were given.

Two further experiments from the same series are described below. These were performed on shoots which had been vaselined and slit the previous evening, and which had had time to settle down to a constant rate of transpiration.

<sup>1</sup> The transpiration at first may be influenced by increased absorption by the newly exposed end of the stem. In the case of branches whose water-content had fallen overnight, this would account for the maximum being reached after an interval. This explanation, however, does not throw any light on the subsequent fall of the rate of water-loss. It was suggested by Priestley (in a discussion at the British Association Meeting in 1923) that in the early morning the intercellular spaces may be somewhat injected, and that therefore in some cases, when the mesophyll is put into contact with the outer air, the meniscus of the intercellular water surface would retire, leaving larger areas of wet cell-wall to increase the amount transpired. This would lead to a temporary increase of water-loss, which would later fall. The long period required to reach the constant rate in the experiments, however, renders this explanation unlikely.



TABLE III.

*Experiment B 14.* July 3, 1923. Ivy, six current year's leaves, prepared July 2. New surface exposed on stem, 11 a.m.

Time. p.m.	Water-loss. mg.	Conditions.
11.30-12 noon	50	Dark.
12.0-12.30	50	
12.30-1.0	50	
1.0-1.30	50	For temperatures see graph.
1.30-2.0	48.5	
2.0-2.30	47.5	
2.30-3.0	47	Light.
3.0-3.30	52	
3.30-4.0	49	
4.0-4.30	49	
4.30-5.0	49	

In Fig. 3 these results are shown graphically.

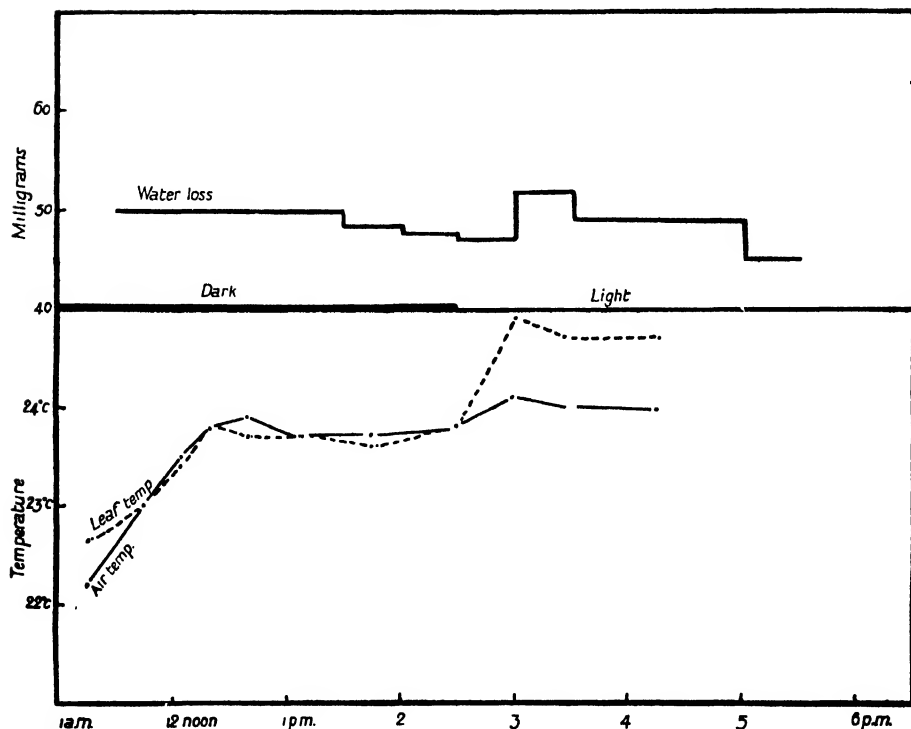


FIG. 3. Expt. B 14. July 3, 1923. Effect of continuous light on transpiration of Ivy leaves, prepared July 2.

TABLE IV.

*Experiment B 16. July 5, 1923. Ivy, vaselined and cut, July 4. Started 11 a.m.*

<i>Time.</i> p.m.	<i>Water-loss.</i> mg.	<i>Conditions.</i>
11.45-12.15	50	Dark.
12.15-12.45	51	
12.45-1.15	51	For temperatures see graph.
1.15-1.45	49	
1.45-2.15	50	
2.15-2.45	52	Light.
2.45-3.15	54	
3.15-3.45	52	
3.45-4.15	50	
4.15-4.45	46	
4.45-5.15	48	

In Fig. 4 these results are shown graphically.

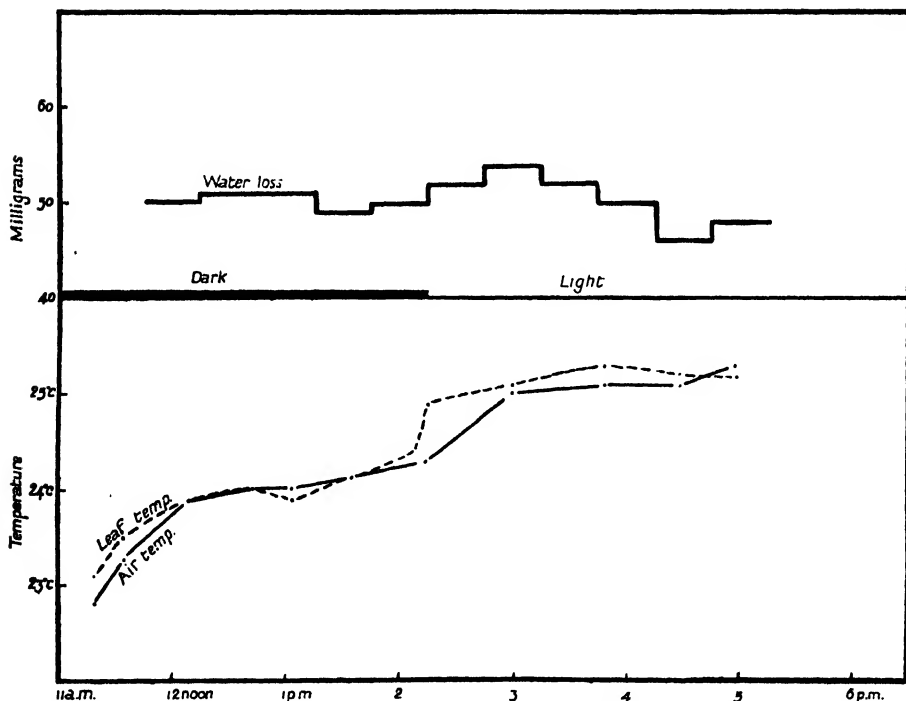


FIG. 4. Expt. B 16. July 5, 1923. Effect of continuous light on transpiration of Ivy leaves, prepared July 4.

Examination of the graphs of water-loss from leaves which have been allowed to attain a constant rate of transpiration after slitting shows that light of the intensity used in these experiments has a direct effect, but

only a slight one, on the rate of water-loss from the mesophyll. The results are very different from those obtained by Darwin; they show that the large differences between the rates of water-loss in dark and in light during the period following the slitting of the leaves in his experiments were not mainly due to the action of light, but to the abnormal conditions caused by the preparation of the leaves for the experiment.

The actual increase of transpiration rate from the mesophyll due to light of the intensity employed is shown by the series of experiments of which the four above experiments are examples. The increases were as follows: 0-1 per cent., three cases; 1-2 per cent., four cases; 2-3 per cent., one case; 3-4 per cent., three cases; 4-5 per cent., six cases; and in one case an increase of 8 per cent. was found.

The leaf temperature usually rose on illumination by an amount varying between  $0^{\circ}$  and  $1^{\circ}$  C. This increase would raise the vapour tension of the water in the mesophyll enough to cause an increase in the transpiration equal to approximately 1 per cent., so that a small increase in transpiration of the order of 1 per cent., due to the warming of the leaf, should be subtracted from the total observed transpiration rate in light. This leaves a quantity of the order of 4 per cent., which is the actual increase in the rate of water-loss due to the light radiation, apart from any heating effect.

The above experiments were carried out with a light of constant intensity. A definite increase in transpiration having been established, however, it was clearly of importance to study the effect of various other intensities of light upon the mesophyll. A further series of experiments, therefore, was undertaken, in which the intensity of the light falling upon the leaves under observation could be varied, the other conditions being kept constant, as in previous experiments.

The preliminary treatment of the leaves—blocking the stomata with vaseline and exposing the mesophyll by slitting—was the same as that used for the experiments described above. A more powerful light, however (a 200-watt gas-filled lamp), was employed instead of the weaker lamp. This lamp hung counterpoised so that it could be rapidly moved up or down above the case holding the automatic balance. The actual amount of energy falling on the leaves was measured by a sensitive Moll thermopile (Cambridge Instrument Co.) placed inside the case, and attached to a horizontal arm which could be raised or lowered to the plane of the specimen, and by means of which it could be swung outwards into the light, or inwards against the side of the case, where it rested under a small blackened shelf when not in use. The position of the thermopile under this shelf gave the 'dark' zero reading, which was taken at frequent intervals. The light measurements were made by means of a moving coil galvanometer of 2.4 ohms resistance.

The relative light energy was read off directly on the scale, the reading in the dark being taken as zero. The thermopile being always in the case and the readings in the illuminated position being taken quickly, accurate results were obtained.<sup>1</sup>

As a check on the behaviour of the leaves, the rate of water-loss from a 'Piche' atmometer was observed *pari passu* with that from the leaves. In no case was any increase observed except with intensities of light high enough to increase the temperature.

For the series of experiments now to be described *Eupatorium adenophorum* was selected rather than Ivy, which was previously used, because a number of plants from the same batch of cuttings and with the same history were available.

It was decided to give each shoot five or six half-hour periods of darkness, followed by a similar number of periods of light of one intensity only. This procedure would enable the significance of the results to be determined, and from several such results the average percentage increase in water-loss caused through that particular intensity might be found. Another lot of shoots would yield an average increase for another intensity of light, and so on. Since all the shoots had the same history, it was hoped to plot a curve of transpiration increase against light intensity for this species of *Eupatorium*. The results of investigations carried out upon six shoots having the same history are shown in Table V. During these experiments no change was made in the apparatus at all, the conditions remaining constant throughout, and the light being fixed at a distance of about 30 in. above the shoot.

TABLE V.

Expt. No.	Plant.	Air Temp. °C.	Humi- dity %.	Thermo- pile Reading.	Water-loss.		Increase %.
					Dark. mg. per $\frac{1}{2}$ hour.	Light.	
E 1	<i>Eupa- torium</i>	26.2	50	25 cm.	48.0 $\pm$ 0.38	50.4 $\pm$ 0.42	5.0 $\pm$ 1.18
E 2	"	26.0	50	25 cm.	65.1 $\pm$ 0.45	71.2 $\pm$ 0.47	9.35 $\pm$ 1.0
E 3	"	26.0	50	25 cm.	50.7 $\pm$ 0.21	54.3 $\pm$ 0.34	7.1 $\pm$ 0.8
E 4	"	26.0	52	25 cm.	40.3 $\pm$ 0.21	41.1 $\pm$ 0.29	1.5 $\pm$ 0.89
E 5	"	26.1	50	25 cm.	65.0 $\pm$ 0.11	67.8 $\pm$ 0.27	4.15 $\pm$ 0.45
E 6	"	26.1	52	25 cm.	63.1 $\pm$ 0.25	66.3 $\pm$ 0.21	5.1 $\pm$ 0.52

In the above table the rate given for water-loss in dark or light is the mean value for several half-hour periods, five or six in most cases. The

<sup>1</sup> An idea of the sensitivity of the thermopile and an indication of the intensity of light used in all these experiments may be gained from the fact that the deflexion obtained by exposing the thermopile to a dull sky in mid-December was 13.2 cm.

probable errors have been calculated on this number of values, which, of course, is rather small; they show, however, that the results are significant. In the last column the difference between dark and light rates of water-loss is brought to a percentage of the 'dark' rate, and the probable error of the difference calculated in the usual way.

This table shows clearly that each plant—probably each leaf—gives a different response to any one intensity of light. It is impossible, therefore, without very great labour to obtain by this method responses with a small enough probable error to allow of comparison of the effect of different intensities of light.

An alternative method was therefore adopted, and a series of experiments begun in which each shoot was exposed to all the intensities of light to be studied, and the water-loss measured. A possible objection to this method is that change may occur in the mesophyll during the course of the experiment. In the experiments already shown in Table V the duration of each experiment was from five to six hours. In the series about to be described next, the experiments lasted much longer, because of the greater number of changes of light to which each shoot was subjected. Several experiments were, in fact, spoiled by the wilting of the specimen, but, since in most cases the experiment ended with a fairly long exposure to a given light intensity, or with a period of darkness, any such wilting or any irregularity in the rate of water-loss could easily be detected.

During these experiments, as in the previous series, the temperature was maintained near 25° C. and the humidity at about 50 per cent. saturation. The exact conditions are stated in each experiment.

The details of manipulation for this series of experiments were as follows: the leaves were vaselined and slit the previous evening, and the shoots were kept in the balance case overnight to avoid any large temperature changes at the beginning of the experiment. Rather than attempt to force all the leaves of one shoot into the same plane, two shoots were usually employed, each with two or three large leaves, the top and smaller leaves of each shoot having been removed some days before. In this way it was possible to have a number of large leaves mounted less than 1 inch apart. In the morning a new surface was exposed at the cut end of the stem, the shoot placed upon the balance pan, and the thermo-couple by means of which the leaf temperature was determined was adjusted in the tissue of one of the leaves. The balance case was then closed and darkened.

After half an hour's run to allow the conditions within the case to become constant, a transpiration record was begun in the dark. When the desired period, usually an hour, had elapsed, the 'dark' reading of the thermopile was taken, the top of the balance case was uncovered, the thermopile swung out, and the lamp switched on and adjusted to the height required to give the desired thermopile deflexion on the scale.

A quarter of an hour was then allowed to elapse for the new rate of water-loss to establish itself, and a record taken for this intensity, after which the light was again adjusted, and so on. The thermopile 'dark' reading was made immediately before the beginning of each light period.

Two experiments of a series of about twenty are shown in detail in Tables VI and VII, and graphically in Figs. 5 and 6. The results of the other experiments appear in Table VIII.

TABLE VI.

*Experiment F 12. Ivy. Three large leaves, vaselined and slit previous evening. Periods of 1 hour, with 10-minute intervals.*

<i>Thermopile Reading. cm.</i>	<i>Air Temp. °C.</i>	<i>Leaf Temp. °C.</i>	<i>Humidity %.</i>	<i>Water loss. mg. per Hour.</i>
0 (dark)	26.6	26.4	55	60
8.3	26.6	26.4	55	60
13.3	26.8	26.4	56	59
19.3	26.4	26.8	55	61
25.3	26.8	26.7	55	65.5
34.3	26.5	26.7	56	66
37.3	26.6	26.8	55	65

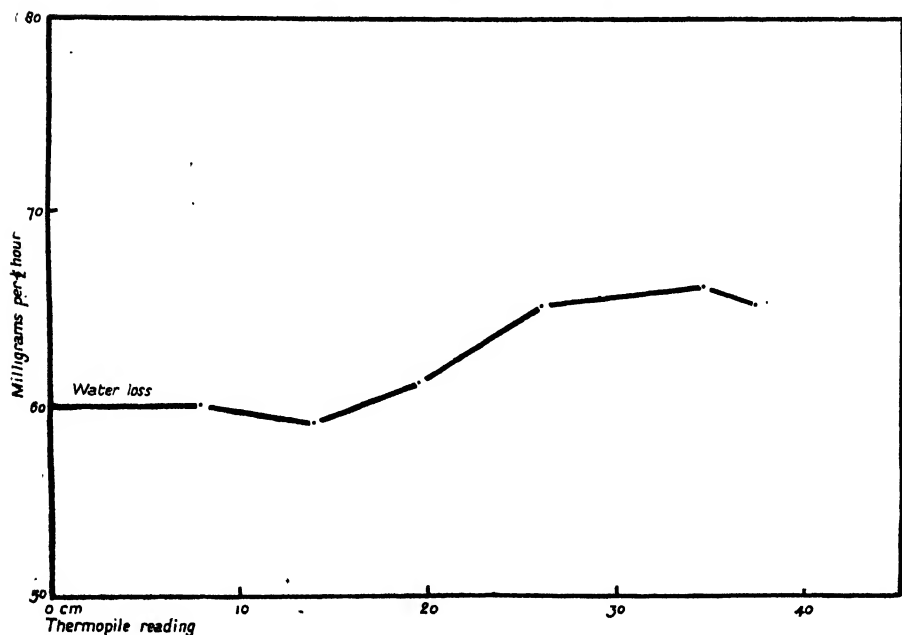


FIG. 5. Expt. F 12. Effect of increasing intensities of continuous light on transpiration of Ivy leaves. Each intensity given for one hour, with 10-minute intervals.

TABLE VII.

Experiment F 21. *Eupatorium adenophorum*. Four leaves, vaselined and slit previous evening. Half-hour periods, with 15-minute intervals.

Thermo- pile Read- ing. cm.	Air Temp. °C.	Leaf Temp. °C.	Humidity %.	Water-loss. mg. per $\frac{1}{2}$ Hour.
0 (dark)	25.0	24.8	61	64
8.5	25.0	24.7	—	64
13.5	25.0	24.8	—	65
17.5	25.2	24.8	60	64
21.5	25.0	25.0	—	66
25.5	25.2	24.8	60	67
29.5	25.0	25.0	59	68
40.5	25.4	25.1	—	68
33.5	25.4	25.5	59	68
-0.5 (dark)	25.2	25.2	—	65

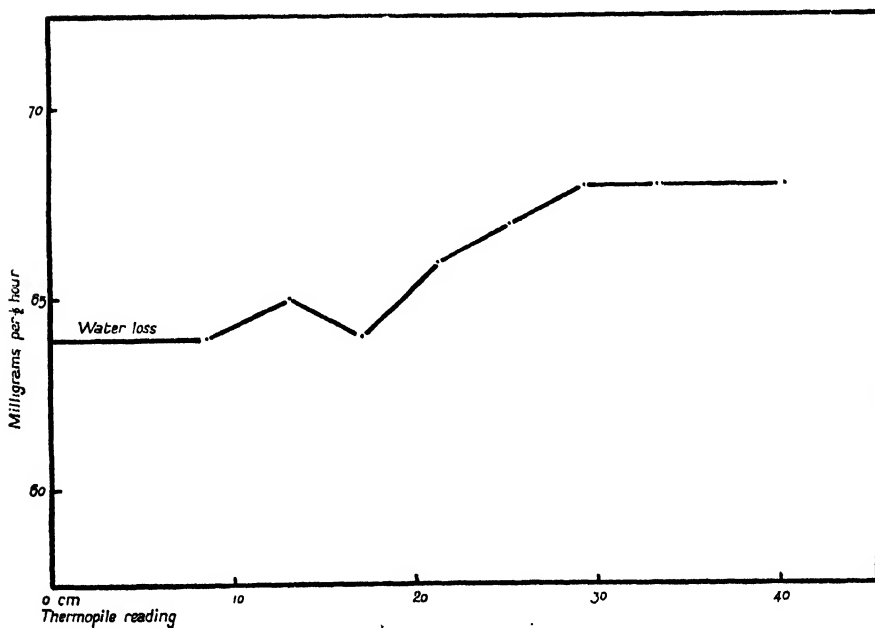


FIG. 6. Expt. F 21. Effect of increasing intensities of continuous light on transpiration of *Eupatorium adenophorum*. Each intensity given for  $\frac{1}{2}$  hour, with 15-minute intervals.

It will be seen from Tables VI and VII and Figs. 5 and 6 that very feeble light has no appreciable effect upon the rate of water-loss from the mesophyll. At a relatively low intensity, however, the rate of water-loss begins to rise until, after a small percentage increase of light intensity, a value is reached which does not go up farther until the leaf temperature, as shown by the thermo-couple, begins to rise and the diffusion gradient steepens.

TABLE VIII.

<i>Expt.</i>	<i>Plant.</i>	<i>Maximum Increase %.</i>	<i>Average Increase %.</i>
F 1	<i>Chrysanthemum</i>	9.1	7.0
2	"	4.9	
4	Ivy	10.1	
5	"	5.8	7.1 ± 0.58
6	"	8.3	
8	"	12.8	
9	"	3.7	
10	"	4.2	
11	"	7.8	
12	"	6.6	
13	"	1.2	
15	"	8.9	
16	"	10.3	
17	"	4.7	5.06
18	"	8.0	
19	<i>Eupatorium</i>	5.1	
20	"	4.2	
21	"	5.9	

Table VIII shows the *maximum* amount of this increase in each experiment in this series, corrected for small changes of temperature or humidity where they have occurred. In these experiments, in the case of Ivy the average increase in light is 7.1 per cent., a slightly higher value than for *Eupatorium* (5.06 per cent.). The two experiments performed upon the garden *Chrysanthemum* (var. *Mme Desgrange*) gave values of the same order as those of Ivy. Several unsuccessful attempts were made to determine more exactly the shape of the transpiration curve at the region of increase, by making a larger number of light changes in that region. However, it is not possible to foretell accurately at what intensity of light the increase of water-loss begins for any individual shoot, and the shortness of the range over which light is effective, the smallness of the differences in water-loss, and the limit of accuracy of the apparatus prevent the relation of light intensity and water-loss being more accurately determined at this point. Greater accuracy might, of course, be obtained by doubling or trebling the period of exposure to each light intensity, but this would extend the duration of each experiment to many hours, and, as was pointed out above, the possibility of change in the mesophyll or of wilting make this inadvisable.

In one or two cases during this part of the investigation a return was



made to a light intensity which had been previously used, after the leaves had been subjected to a higher intensity, to see whether an after-effect could be detected. As far as can be determined no such effect seems to occur, unless it lasts no longer than the short period allowed for the establishment of the new rate of water-loss.

## II. Experiments with Intermittent Light.

A series of experiments was performed to determine what effect intermittent light has on the rate of water-loss, in the hope that it might elucidate in some degree the mechanism of control of water-loss. A disc divided into quadrants, two of which were removed, was rotated between the source of light and the shoot by a small motor having a variable resistance in series, by means of which the speed of rotation of the disc could be varied between about 150 and 1,000 revolutions per minute, giving intermissions of light of from 300 to 2,000 per minute. The leaves were usually subjected to a preliminary period of darkness, then to one of continuous light, and thirdly to periods of intermittent light of varying frequency of interruption, the usual 15 minutes being allowed for establishment of the new rate of water-loss before taking any record.

Two experiments with intermittent light are given in detail below.

TABLE IX.

Ivy. Four large leaves, vaselined and slit the previous evening. Half-hour periods, with 15-minute intervals. Humidity, 62 per cent.

<i>Thermopile Reading.</i> cm.	<i>Intermissions per Minute.</i>	<i>Air Temp.</i>	<i>Water-loss.</i> mg./ $\frac{1}{2}$ Hour.	<i>Increase of Water-loss in Light.</i>
0	(dark)	25.5	134	—
24.5	(continuous)	25.5	160	26 mg.
12.2	300	25.7	149	15
12.5	475	25.7	151	17
12.8	700	25.5	151	17
12.7	1,200	25.5	152	18
—	1,500	—	154	20
12.8	1,800	25.6	154	20

TABLE X.

Ivy. Three large leaves, vaselined and slit the previous evening. One-hour periods, with  $\frac{1}{4}$ -hour intervals. Air temperature, 25.5° C. Humidity, 60 per cent.

<i>Thermopile Reading.</i> cm.	<i>Intermissions per Minute.</i>	<i>Water-loss</i> mg./Hour.	<i>Increase of Water-loss in Light.</i>
0	(dark)	270	—
24.0	(continuous)	294	24 mg.
12.0	350	280	10
12.1	550	281	11
12.1	700	284	14
12.0	1,150	290	20
12.1	1,550	290	20

These two experiments form part of a series of fifteen experiments, from ten of which results similar to the above were obtained. It will be seen from the above tables that when the disc is rotated and the energy received by the leaves thus cut down to half that received during continuous illumination, the increase of water-loss relative to that in the dark falls in the case of slow speeds to a value usually slightly less than half the value for continuous light. With each increase in the number of intermissions, however, the rate of water-loss rises, tending, presumably, towards the value for continuous illumination. Thus with the same total radiation received by the leaf a greater and greater effect is obtained as the periods of darkness are shortened.

In several experiments the rate of water-loss rose only slightly or not at all on increasing the rate of light intermission. The cause of this will be discussed later.

#### *Energy absorbed by the leaves.*

During the investigation tests were made with leaves of Ivy and of *Eupatorium* to find the average amount of absorption of the incident light. Ten leaves were picked at random, and were placed in turn between the thermopile and the source of light. In the case of Ivy, the thermopile reading fell from 19.5 cm. to the average value of 3 cm. This corresponds to an absorption of 84.5 per cent. of the energy received by the leaf. With *Eupatorium*, the thermopile reading fell from 20 cm. to the average value of 4.15 cm. on the interposition of the leaf. This corresponds to an absorption of 78.25 per cent. of the energy. These results are both higher than that obtained by Brown and Escombe for the leaf of *Helianthus*. Neither in these experiments nor in those of Brown and Escombe is any account taken of reflection of light from the leaf surface.

### *III. Effect of Humidity Changes on Transpiration from the Mesophyll.*

Darwin (3) investigated the effect of changes of humidity on the rate of water-loss, using slit leaves. He obtained the changes of humidity by the gradual saturation of the atmosphere in a bell-jar by the plant itself, the changes in humidity being read from a wet- and dry-bulb thermometer. With rapid changes of humidity such as Darwin used, this method suffers from want of correspondence in time between transpiration and absorption. In addition to this, the humidity is changing during each period for which the water-loss is determined. Another method was therefore adopted, that of subjecting the plant to an atmosphere of constant known humidity, then changing the humidity and allowing a new rate of water-loss to be established. This was conveniently done by passing over the plant a stream of air saturated to the required degree by bubbling through a solution of calcium chloride of known vapour tension. One of a short series of experiments on humidity changes is given in detail below.

TABLE XI.

*Experiment C 3.* July 19, 1923. Ivy vaselined and slit July 18.  
New surface of stem exposed.

11.20 a.m.	Started. Humidity, 70 %. Air temperature, 24° C. Leaf temperature, 22.7° C.
2.15 p.m.	Transpiration steady at 122 mg. per 30 minutes.
2.45 p.m.	Humidity changed to 80 %. Transpiration 83 mg. per 30 minutes.

In Fig. 7 these results are shown graphically.

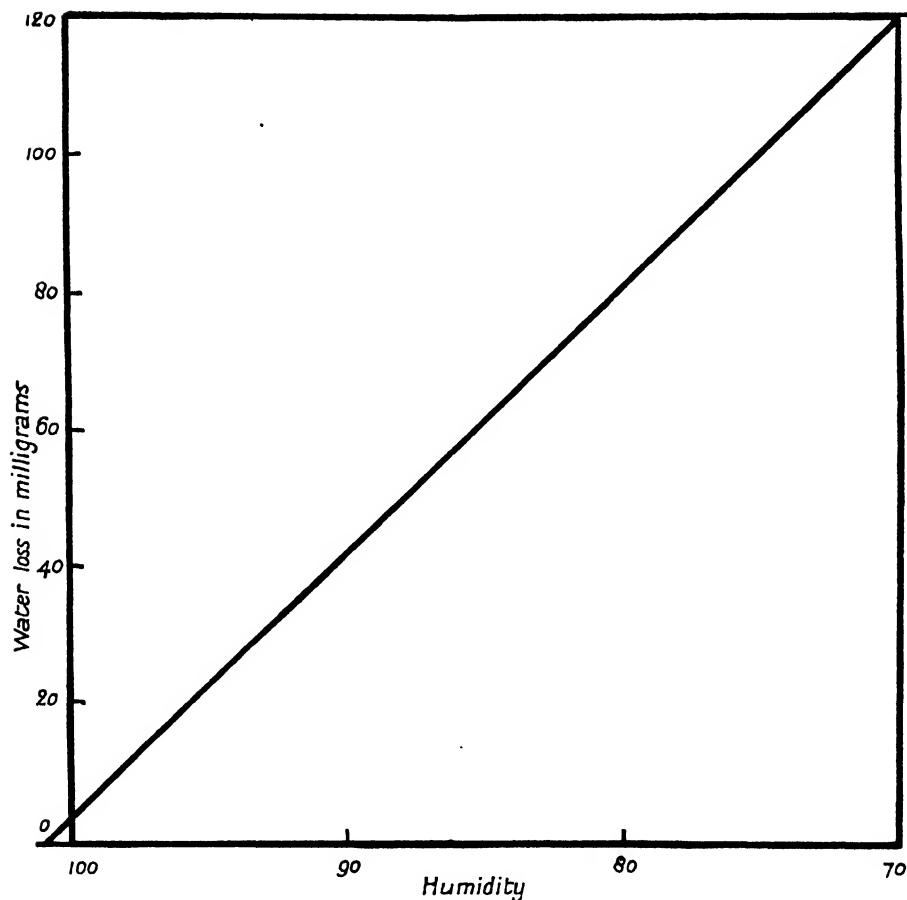


FIG. 7. *Expt. C 3.* July 19, 1923. Effect of changes of humidity on transpiration of Ivy. Leaves prepared July 18.

Darwin found that the relation between transpiration and humidity was a linear one, and these experiments confirm his conclusions with the exception that here the graphs do not cut the *x*-axis so far to the left of the

*y*-axis as in Darwin's case. That is to say, the temperature of these leaves when the atmosphere is saturated is about  $0.4^{\circ}\text{C}$ . above the air temperature, whereas Darwin's estimate is about  $1^{\circ}\text{C}$ .

#### IV. *The Effect of the Water-content of the Mesophyll on the Rate of Transpiration.*

A series of experiments was performed with the object of finding whether the water-content of the mesophyll bore any relation to the rate of transpiration. The work was carried out at the Chelsea Physic Garden, and, instead of using the automatic apparatus, the air-flue designed by Blackman and Knight (1) was used, so that sudden changes in the evaporating power of the air could be obtained without change of air temperature or humidity. The technique is fully described in the authors' paper, but it may be mentioned that the flue is so constructed that a current of air free from eddy currents is drawn past the plant by a governed motor running at a constant speed. By altering the speed of the air current, the evaporating power and hence the transpiration rate can be changed. If, therefore, a plant whose leaves have been vaselined and slit be fitted to a simple potometer of the burette type, and be subjected to an air current of high velocity, it will be found that transpiration will exceed absorption, i. e. that the water-content of the leaves will decrease. Hand in hand with this goes a drop in the transpiration rate, which ultimately falls approximately to the absorption rate. If at this point the fan be stopped, the transpiration rate will drop sharply, and will fall well below the absorption rate; the water-content of the leaf will therefore increase. If the fan be again started at the previous speed, the plant will be subjected to the same conditions as before, and the effect on the transpiration rate of the increased water-content can be determined.

One experiment of a series of about twenty will serve to show the effect of increased mesophyll water-content on the rate of transpiration. The plants investigated in this series of experiments included Ivy, Lilac, *Crataegus coccinea*, *Physostegia* sp., and *Eupatorium adenophorum*.

As is shown below, an untreated shoot was sometimes included in the experiment, but, as the experiments were performed in diffuse daylight (and stomatal changes were therefore possible if light fluctuations occurred), and as the number of readings falling due each half-hour was uncomfortably large, it was usually omitted. As in Knight's experiments (6), the atmometer was used as a check on the constancy of the evaporating power of the air stream.

It will be seen from the graph that the transpiration curve from both the slit and the intact shoots undergoes a gradual fall to an amount roughly corresponding to the amount of intake of water, which remains relatively steady.

TABLE XII.

Experiment D 11. August 28, 1923. *Eupatorium adenophorum*. Two shoots, one vaselined and slit, Aug. 27, fitted to potometers and put into the flue with an atmometer. Air speed, 20 metres per minute. Air temperature,  $16^{\circ} \pm 0.2^{\circ}$  C. Humidity, 62 per cent.

Time 30 Mins. Ending	Atmometer, loss. mg.	Vaselined Shoot.		Intact Shoot.	
		Water- loss.	Poto- meter.	Water- loss.	Poto- meter.
12.35 p.m.	440	170	120	420	180
1.5	450	150	110	350	180
1.35	480	130	110	230	200
2.5	480	130	110	230	200
2.35	440	130	120	180	210
3.5	470	120	120	170	190
3.35	470	120	110	150	200
4.5	470	110	130	150	170
4.35 fan stopped	270	80	120	90	180
5.5 "	280	80	110	100	170
5.35 fan restarted	480	130	120	180	180

In Fig. 8 these results are shown graphically.

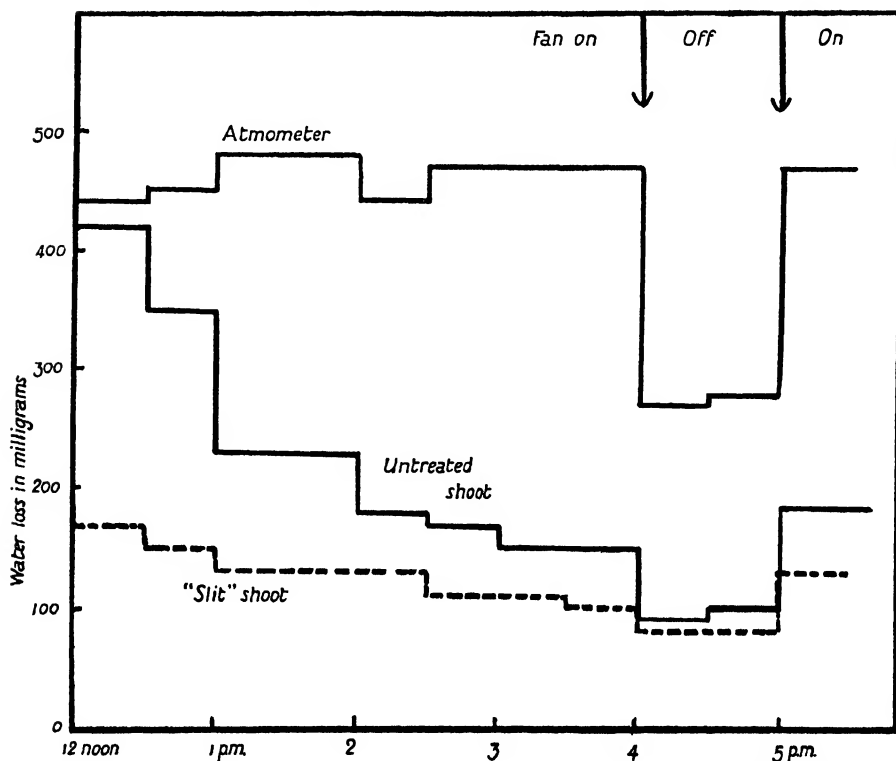


FIG. 8. Expt. D 11. August 28, 1923. Effect of changing water-content of the mesophyll on transpiration of *Eupatorium adenophorum*.

On stopping the fan, the evaporating power of the air is lessened, and the transpiration rate falls accordingly. After an hour's interval, on restarting the fan, the transpiration for the next half-hour is found to exceed that of the half-hour immediately preceding the stoppage of the fan, the increase being due to the only factor that has been altered, the increased water-content of the mesophyll.

These results confirm Knight's conclusions from experiments carried out with an intact leaf and with constant stomatal aperture in darkness, and extend the number of species in which this response to increased leaf water-content has been observed.

### *Leaf Temperatures.*

Examination of the leaf and air temperature records of the light and humidity experiments reveals an interesting point. It will be seen from the transpiration-humidity graph on p. 525 that the leaf temperature, when the air is at 100 per cent. saturation, is above that of the outside air by an amount of  $0.4^{\circ}\text{C}$ . This fact is often taken to mean that under all ordinary conditions the leaf is maintained above atmospheric temperature by its heat of respiration. The case in question is, however, a very special one. At less than 100 per cent. saturation, the leaf temperature is determined by the evaporating power of the air, and in experiments with such humidities, where a gentle current of air was passing over the leaf, its temperature was usually found to be below that of the surrounding air, and to be influenced by changes in its power of evaporation. An actual example will demonstrate this. In one experiment, with a constant rate of movement of air, the leaf temperature was  $1.3^{\circ}\text{C}$ . below that of the air ( $22^{\circ}\text{C}$ .) at a humidity of 70 per cent. On raising the humidity to 80 per cent., the leaf temperature rose  $0.5^{\circ}\text{C}$ ., and became steady at  $0.8^{\circ}\text{C}$ . below the temperature of the surrounding air.

As these leaf temperatures are dependent to some extent on the rate of air movement round the leaves, the actual change of temperature per degree of humidity cannot be calculated for all conditions. It seems probable from the data, however, that in the case of Ivy, the heat of respiration is just balanced by the heat-loss of evaporation (i. e. the leaf usually reaches atmospheric temperature) at about 95 per cent. humidity, when the temperature is about  $20^{\circ}\text{C}$ ., the air movement gentle, and the light diffuse. In sunlight, of course, the heat energy received by the leaf will more than counterbalance the evaporation heat-loss, and, except in cases of rapid air movement round the plant, the temperature of the leaf will be above that of its surroundings; but it is important to note that, under conditions of diffuse light, the leaf is often cooler than the air into which it is transpiring.

#### DISCUSSION OF RESULTS.

From the foregoing data for Ivy, in the absence of stomatal control, it will be clear that light has not the marked effect on water-loss from the mesophyll that has been claimed. In the preliminary series of experiments a slight increase of the order of 5 per cent. is found, however, and this amount cannot be accounted for by the higher temperature of the leaf in light, for, under the conditions of the experiments, the increased temperature would only bring about an increase of transpiration of about 1 per cent. The interesting result therefore emerges that, apart from the heat effect, there is a slight direct effect of light on the mesophyll leading under the conditions of the experiments to an increase of water-loss of about 4 per cent.

The further results of the experiments with continuous light confirm those of the preliminary series. They indicate, however, that the increase in water-loss from the mesophyll produced by light varies greatly between plant and plant, a difference that probably extends to the individual leaves. The values range from 1.5 per cent. to 9.35 per cent. The conditions of temperature, humidity, and air movement were unchanged during the course of the experiments, so that this variation in the rate of water-loss must be due to the conditions obtaining in the leaf itself. It has been shown in this paper by the writer, and elsewhere by others (4), that the transpiration rate is dependent upon the water-content of the mesophyll.

As far as has been observed, the age of the leaf, at least over the period of late summer when most of the experiments were performed, does not seem to affect to any appreciable extent the magnitude of the response.

In the last series of experiments with continuous light, in which gradually increasing light intensities were used, no increase in the rate of water-loss seems to take place until a certain minimum value of light is attained, then a relatively rapid increase of water-loss takes place over a short range of increasing light intensity. This rise soon ceases, however, and the rate of water-loss remains steady until, with increasing light intensity, the temperature of the leaf begins to rise. A 'light' rate and a 'dark' rate of water-loss have thus been established, but owing to the small difference between these two rates in comparison with the experimental errors involved, it was not found possible to work out fully the relation of different intensities of light to this increase in water-loss. There is some indication, however, that the relation is a linear one (*vide* Figs. 5 and 6). The light intensity at which the response to light appears is very different for different plants.

The results of the experiments with intermittent light are of marked interest. Leaves receiving alternate light and dark periods of 0.2 sec. show an increase in the rate of water-loss equal to about half the increase in *continuous* light of the same intensity. With an increased speed of rotation of the disc, however, the rate of water-loss goes up, and it is clear that light of the same intensity is more efficient in accelerating water-loss with rapid intermissions than with slow intermissions. It would seem that the conditions favourable to increased water-loss, induced by illumination, are produced almost at once, but revert to the 'dark' conditions at a slower rate. On this assumption the shorter the periods of darkness, the greater will be the fraction of each dark period during which the light conditions persist; in other words, the higher the speed, the longer the total period of 'light' conditions. These results are similar to the results obtained by Warburg (9) in his experiments on the assimilation of carbon dioxide by green algae, although, of course, the mechanisms involved can hardly be comparable.

In some of the experiments with intermittent light no rise in the rate of water-loss was observed, although increase occurred in continuous illumination. This is presumably due to the fact that while the continuous illumination caused a certain amount of response, the reduction of the radiant energy to half left it below the minimum required for response. On the other hand, one might expect also that, if the light intensity in continuous light were considerably greater than that required to give the maximum increase in water-loss, this latter rate might be reached at the higher speeds of the rotating disc. This condition, however, was not observed during the course of these experiments, perhaps because the continuous light intensities were kept as low as possible for fear of warming the leaf.

The effect of changes of humidity on the rate of transpiration shows that for higher humidity values the surface of the cells acts as a damp surface in a purely physical way, since the graphs of both these experiments and those of Darwin can be shown to obey, within fairly narrow limits, the equation for change of rate of water-loss with change of humidity:

$$E_1 = E \left( \frac{S_{T_1} - \frac{y}{100} S_h}{S_T - \frac{x}{100} S_t} \right),$$

where  $E$  = rate of evaporation when  $S_T$  is the saturation vapour pressure of the air at leaf temperature  $T^\circ$ ;  $\frac{x}{100} S_t$  is the percentage of the saturation water vapour pressure at air temperature  $t^\circ$ ;  $E_1$  = rate of evaporation when  $S_{T_1}$  is saturation vapour pressure of the air at leaf



temperature  $T_1^\circ$ , and  $\frac{y}{100} S_{t_1}$  is the percentage of the saturation water-vapour pressure at air temperature  $t_1^\circ$ .

It must be noted that, while the curves of humidity and transpiration admit of interpolation between 100 per cent. and, say, 60 per cent. humidity, one cannot legitimately extrapolate for low humidities, since it is probable that near the point of wilting other changes will affect the rate of evaporation from the cell-wall.

Experiments on change of transpiration rate with temperature changes have not been carried out, as no simple humidity regulator which will act uniformly at changing temperatures appears to be available; the hair hygrometer regulator will work satisfactorily only at relatively constant temperatures.

No experiments have been performed on the effect of changing concentrations of carbon dioxide on the rate of water-loss from the mesophyll. It might be suggested that in darkness the increasing concentration of this gas in the intercellular spaces of the leaf could affect the transpiration rate, but, since the increase in the amount of carbon dioxide establishes a steeper diffusion gradient, it is unlikely that the concentration ever rises enough to affect the rate of water-loss to any appreciable extent.

A general survey of the results obtained from the experimental work on water-loss confronts us at once with the complexity of the conditions concerned. The discovery of the fact that light causes an increase in the water-loss from the mesophyll immediately raises the question of the mechanism of that increase. The chief factors which might influence the rate of water-loss, apart from alteration in the evaporating power of the air, would appear to be (1) changes in the water-content of the mesophyll; (2) changes in the protoplasm of the mesophyll cell; and (3) changes in the mesophyll cell-wall itself. The mesophyll water-content would naturally influence the transpiration rate, and it is no doubt partly by the interaction of this factor with external ones that the normal transpiration rate is determined. It is probable that differences in the water-content of the different leaves, and of leaves of different specimens, explain the great variations (1.5 to 9.35 per cent.) observed in the light response of the mesophyll. Unless, however, the mesophyll water-content actually itself change with the incidence of light, it cannot be regarded as a cause of a changing rate of water-loss. Of course, it is possible that the water-relations of the cell may be affected by some imbibition changes in the cell colloids caused by the action of light. It is conceivable, also, that the light, as in the case of guard cells, may produce some alteration in the starch-sugar relation; any increase in the concentration of sugar would, however, tend to reduce the rate of water-loss rather than increase it.

The possibility that light may affect the complex cell-wall in such

a manner as to render it more permeable to water, a condition of things which would increase the rate at which water would be available at the outer surface, cannot be excluded. The most probable explanation, however, would seem to be that light causes changes in the permeability of the protoplasm to water. If light reduces the resistance to the passage of water through the protoplasm, the supply available to the cell-wall will be increased, its imbibition will be higher, and the vapour tension at the evaporating surface of the cell-wall will tend to rise. Equilibrium at a more rapid rate of water-loss will then be established.

That increase in the intensity of the light does not continue to increase the rate of water-loss may be due to the fact that higher intensities of light reduce no farther the resistance of the protoplasm to water-loss, or more probably that the resistance of the passage of water of the system cell-wall, plus protoplasm, is mainly due to that of the cell-wall.<sup>1</sup>

The results of the experiments which have just been described suggest two further lines of research. The more important of these is the study of the relation between temperature and rate of water-loss under different intensities of light. Although the results of such an investigation would be of marked interest, yet the small differences in water-loss involved make the prospect of success small with the present technique. The other line of investigation that suggests itself is the effect of intermittent light upon transpiration from the intact leaf, especially in relation to its effect upon the guard-cells.

#### SUMMARY.

In transpiration, the response of the mesophyll to ordinary external conditions is masked by the action of the stomata. The stomata, however, can be rendered inoperative by the 'slitting' method, and so the direct effect of external conditions on water-loss from the mesophyll can be studied.

The large increases in transpiration rate (10 per cent. to 100 per cent.) ascribed by Darwin to the direct effect of light on the mesophyll could not be confirmed.

Electric light of the intensity of diffuse daylight was found to increase the mesophyll water-loss by an amount of the order of 5 per cent., of which about 1 per cent. is a heating effect. A direct action of light on the rate of water-loss from the mesophyll cells has thus been established.

Very feeble light appears to have no appreciable effect upon transpiration, but with increasing intensities the rate of water-loss rises. It soon,

<sup>1</sup> Of course, the supply of water to the cell-wall may be still more complex and of the nature of 'secretion', as Dixon has suggested, but as we have no knowledge of this process, if it occurs, a discussion of it is not profitable.

however, reaches a maximum. In the case of Ivy this maximum is 7.1 per cent. above the rate of water-loss occurring in the dark, and with *Eupatorium adenophorum* the corresponding increase is 5.06 per cent.

Marked differences varying from 1.5 per cent. to 9.35 per cent. were observed in the light response of carefully selected cut shoots of *Eupatorium adenophorum* exposed to the same intensity of light.

With intermittent light a factor other than intensity of light comes into play, a gradual increase in the effect being observed with increase of the rate of intermissions from 300 up to 2,000 per minute, although the amount of radiation falling on the leaf remains the same. An after-effect of the light which continues into the dark period is thus indicated.

The mesophyll reacts to humidity changes in a way similar to that of a damp inert surface. The transpiration-humidity curve is linear, and obeys the saturation gradient equation at least at the higher humidities investigated.

The leaf temperature in Ivy, *Eupatorium adenophorum*, and *Aster* sp. in gently moving air, diffuse light, and laboratory temperature and humidity is below that of the surrounding air. As humidity or light intensity increase, however, the leaf temperature rises to a higher value, which may be above that of the air.

The water-content of the mesophyll is an important factor in determining the rate of transpiration.

The nature of the light response of the mesophyll is discussed. It is suggested that the most plausible explanation is the effect of light on the permeability of the protoplasm to water.

In conclusion, the writer wishes to tender his thanks to Professor V. H. Blackman for his kindly interest and criticism.

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## Notes on the Genus *Meconopsis*, with some Additional Species from Tibet.

BY

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With Plate XVI.

IN 1924, through the kindness of the Indian and Tibetan Governments, I obtained permission to travel and collect plants in Tibet. The Government Grant Committee of the Royal Society and the Trustees of the Percy Sladen Memorial Fund financed me, and Lord Cawdor, for whom permission was also obtained through the Governments concerned, volunteered to accompany me.

Our objective was the gorge of the Tsangpo-Dihang and the Assam Himalaya, about which practically nothing was known botanically ; though the expedition of Majors Bailey and Morshhead in 1913 had indicated a very rich flora in south-eastern Tibet.

We left Darjeeling on March 16, 1924. The route followed was via Sikkim and the Chumbi valley to Gyantse, thence along the southern shore of the Yamdrok Tso to Tsetang on the Tsangpo, which we followed eastwards for about 200 miles to the head of the great gorge. Here we botanized for six months, crossing the Himalayan range to the south, and the Salween divide to the north. In November and December we made our way through the Tsangpo gorge, northwards up the Po-Tsangpo, and westwards by the China-Lhasa road back to Tsetang. From Tsetang we turned due south, following the 92nd meridian through eastern Bhutan, whence Assam was reached on February 23, 1925.

The plant collection was made almost entirely in the Tsangpo valley, and on the ranges to north and south of the gorge, between the parallels of  $29^{\circ} 45'$  and  $30^{\circ} 0'$ , and the meridians of  $93^{\circ} 30'$  and  $95^{\circ} 30'$ .<sup>1</sup>

The following species of *Meconopsis* were collected :

K. W. 5627. Fruit only. Allied to *M. integrifolia*, Lung La, Tibet (lat.  $29^{\circ} 30'$  N., long.  $92^{\circ} 30'$  E.), 15,000 ft. (27.4.24.) Plant of 2 ft. ; the

<sup>1</sup> See map, Geographical Journal, Feb. 1926.

main stem bears a number of 1-flowered pedicels. Stigma 5-rayed. Fruit opening by 5 valves. On boulder screes.

K. W. 5628. *M. horridula*. Fruit only. Lung La, Tibet, 14,000 ft. (27.4.24.) On earth and gravel slopes under rocks. (See Nos. 5751, 6006.)

K. W. 5683. *M. Baileyi*. Tsangpo valley (lat. 29° 30' N., long. 94° 30' E.), 9,000–10,000 ft. (18.5.24.) In *Picea* forest, scarcely in flower. (See No. 5784.)

K. W. 5716. *M. simplicifolia*. Tsela Dzong, Tsangpo Valley, 13,000 ft. (30.5.24.) Plants in bud. Grows 2–3 ft. high; under rocks on the southern wind-swept barren flank of the hill.

K. W. 5717. *M. Prainiana*. Tsela Dzong, 13,000–14,000 ft. (30.5.24.) Fruit only; = No. 5909. Grows 3–3½ ft. high, bearing about 20 flowers on 6 in. pedicels. On the windy side of the hill, amongst boulders and dwarf *Rhododendron*, with the last species.

K. W. 5737. *M. simplicifolia*, var. *Baileyi*. Temo La, 13,000–14,000 ft. (5.6.24.) Flowers sky blue, fragrant. On open *Rhododendron* moorland, on the lee side. Common.

K. W. 5749. *M. pseudo-integrifolia*, variety? Temo La, 14,000 ft. (7.6.24.) Plant of 30 in. bearing as many as 10–12 flowers, each 4–5 in. in diameter; petals sulphur; anthers orange. The central stem bears a number of axillary 1-flowered scapes, which are sometimes as tall as the main stem, several usually springing from the same level. Capsule opening to half its length. On open *Rhododendron* moorland. Common.

K. W. 5750. *M. impedita*, var. *Morsheadii*. Temo La, 14,000 ft. (7.6.24.) On boulder screes. Common. (Fruit only: see No. 5808.)

K. W. 5751. *M. Cawdoriana*, sp. nov. *Aculeatae*. Temo La, 14,000 ft. (7.6.24.) Fruit only. On steep windy grass and rock slopes, facing south, growing with No. 5750. Nyima La, 15,000 ft. (20 miles east of Temo La.) (21.6.24.) In flower.

The following is a brief description: plant of 12 in. Central stem 0, or formed by the agglutination of several originally distinct basal 1-flowered scapes. Leaves irregularly pinnatifid, lobes narrow-obtuse. Scapes numerous, distinct, 1-flowered, the flowers half-nodding. Petals 4, sky blue. Style  $\frac{7}{16}$  in. long, green. Stigma green. Resembles *M. horridula* in habit, but the plant is less prickly and the scapes fewer; also flowers are fragrant, and petals 4, instead of 6–8.

K. W. 5766. *M. simplicifolia*, variety? Temo La, 13,000 ft. (10.6.24.) Flowers cream or ivory white, fragrant; otherwise like *M. simplicifolia*. Very rare.

K. W. 5784. *M. Baileyi*. Lunang, 11,000 ft. (15.6.24.) Very common on both sides of the Tsangpo, in woods.

K. W. 5808. *M. impedita*, var. *Morsheadii*. Nyima La, 14,000–15,000 ft. (20.6.24.) No. 5750 in flower. Petals 6–7, dark blue or violet. Anthers

cream, filaments violet, ovary green, style dark violet, stigma cream. On alpine turf slopes and earth screes. Very common on both sides of the Tsangpo, but particularly on the north bank.

K. W. 5855. *M. simplicifolia*, variety? Doshong La, Eastern Himalaya, 12,000 ft. (25.6.24.) Flowers wine coloured, with scent of Shirley Poppy; otherwise like *M. simplicifolia*. In sheltered situations amongst alpine thickets. Common, but local.

K. W. 5909. *M. Prainiana*, sp. nov. Aculeatae. Temo La, 15,000 ft. (7.7.24.)

The following is a brief description: plant of  $2\frac{1}{2}$ – $3\frac{1}{2}$  ft. covered with colourless prickles. Basal leaves, narrow lanceolate to linear, obtuse, shortly petiolate, margin sometimes slightly wavy; blade 4–5 in. long,  $\frac{1}{2}$ – $\frac{5}{8}$  in. wide. Stem leaves smaller, acute, sessile. Flowers numerous, axillary, on pedicels 4–6 in. long. Petals 4, pale blue, broadly obovate, 1 in. long,  $1\frac{1}{4}$  in. wide. Stamens numerous, multiseriate; anthers orange, filaments violet. Fruit ovoid,  $\frac{3}{4}$  in. long, opening by 6–7 valves to half its length. Style  $\frac{1}{2}$  in. long, green. Stigma green. Differs from *M. latifolia* in its proportionately longer style (almost as long as the capsule) and green stigma; and from *M. sinuata* in its capsule—obovoid instead of obconic—and foliage.

K. W. 5910. *M. brevistyla*, variety? Temo La, 16,000 ft. (8.7.24.) Not yet in flower. Plant of 9 in., the main stem ending in a flower and bearing one or two axillary flowers on simple pedicels. On the highest cliffs and screes at the uttermost limit of flowering plants. (See Nos. 5984, 6125, 6213.)

K. W. 5984. *M. brevistyla*, variety? Nam La, Eastern Himalaya, 16,000 ft. (24.7.24.) No. 5910 in bloom. Petals 6–8, cream or sulphur, slightly fragrant; filaments cream; anthers orange. Stigma sessile, hollow, receptive only on the inside, opening after the flower opens, and exposing 6 divergent decurrent rays. On high glacier moraines.

K. W. 6006. *M. horridula*? Nam La, 15,000 ft. (26.7.24.) Petals 4, purple. Dwarf plant of 6–9 in. Filaments purple, anthers orange. Style green, very short; stigma dark purple. In crevices of gneiss cliffs. Just opening.

K. W. 6038. *M. Florindae*, sp. nov. Cumminsia. Tra La, 11,000 ft. (2.8.24.)

The following is a brief description: biennial. Root small, carrot-like. Stems simple, 1–3, about 12 in. high. Leaves linear-lanceolate, obtuse, margin wavy, or slightly (and irregularly) lobed, glabrous, sometimes pinnatisect. Radical leaves petiolate, 5 in. long,  $\frac{3}{4}$  in. wide; stem leaves sessile, the largest 3–5 in. long,  $\frac{3}{4}$  in. wide. Main stem ending in a flower, other flowers few on simple axillary pedicels,  $2\frac{1}{2}$ –3 in. long, which are bristly below the flowers. Flowers nodding. Sepals 2, ovoid, bristly. Petals 5–7,

rarely 4, pale yellow, obovate, 0.9 in. long, 0.5 in. wide, margin irregularly serrate or slightly waved. Stamens numerous, multiseriate; filaments colourless, 0.4 in. long; anthers pale orange. Ovary 0.5 in. long, green; style 0.25 in. long, pale green; stigma rounded, yellow. Fruit narrow-obovoid, opening to half or three-quarters its length by 5 valves.

K. W. 6096. *M. horridula*. Nambu La, Tongkyuk, 13,000–14,000 ft. (15.8.24.) Petals numerous, sky blue, with the sheen of Japanese silk. Filaments sky blue, anthers bright yellow, style green, stigma purple. Stem purple, prickles pale green. On earth banks. (See No. 6126.)

K. W. 6125. *M. brevistyla*, variety? Pasum Kye La, Tsangpo-Salween divide, 16,000–17,000 ft. (25.8.24.) Almost over. On screes and boulder slopes. (See Nos. 5910, 6213.)

K. W. 6126. *M. horridula*. Pasum Kye La, Tsangpo-Salween divide, 15,000–16,000 ft. (25.8.24.) Dwarf plant bearing many (sometimes 30 or more) basal 1-flowered scapes. Petals 6–8, dark blue, inclined to purple (cf. No. 6096), sometimes sky blue. Filaments blue, anthers orange; stigma crimson, with colourless papillae; style purple. Scapes purple, beset with colourless prickles. Abundant on alpine turf slopes, old moraines, earth banks, &c. A good many flowers surviving, though long past its prime.

K. W. 6170. *M. horridula*, var. *racemosa*. Tro La, Tsangpo-Salween divide, 13,000 ft. (30.8.24.) Petals 6, sky blue, inclining to purple. Filaments blue, anthers orange, stigma purple, style and ovary green. There is always a central racemose stem, and usually a few basal 1-flowered scapes as well. On gravel banks.

K. W. 6206. *M. Florindae*. Tra La, 11,000 ft. (27.9.24.) Fruit of No. 6038. Seeds now ripe.

K. W. 6213. *M. brevistyla*, variety? Temo La, 16,000 ft. (2.10.24.) Fruit of Nos. 5910, 5984, 6125. Style 0. Ovary  $1\frac{1}{4}$  in. long, densely covered with orange hairs.

K. W. 6245. *M. simplicifolia*, variety? Doshong La, 12,000 ft. (23.10.24.) Fruit of No. 5855? The style is appreciably longer than in *M. simplicifolia* from the Temo La (No. 5737); also this species produces twice as many scapes as the latter.

K. W. 6259. *M. lyrata*? Doshong La, 13,000 ft. (25.10.24.) Seed only collected. Slender plant of 6–10 in. with long slender capsule, the tip of which was observed projecting through the snow. Like No. 6038 in appearance, but growing on steep alpine turf slope. Only two plants seen.

The classification proposed by Sir David Prain is followed here, with a few suggested alterations, necessitated by increased knowledge.

Sir David Prain divides the genus into two sections—*Eumeconopsis*, plants with simple hairs, setae, or prickles, and *Polychaetia*, with barbellate hairs. So far so good. In the living plant, or with ample dried material,



the distinction is easily observed with a good pocket-lens. With fragmentary herbarium material, or with old specimens, it is more difficult because there may be no hairs to observe, or the barbs may be worn off. Moreover, certain parts of the plant, at least when not mature, may not develop the characteristic barbellate hairs. These two sections are further subdivided into a number of groups or aggregates, most of which are fairly distinct.<sup>1</sup>

The species themselves, however, are often ill defined. Characters used in the subdivision of the two sections are: habit and inflorescence; flower colour—whether belonging to the yellow-flowered or to the blue-flowered group; whether annual, biennial, or perennial.

Characters used in the discrimination of species, in addition to the above, are: shape of leaf; colour of anthers; shape of capsule and its method of dehiscence; relative length of style; number of petals; as in any other large genus. Several of these characters are of little diagnostic value by themselves, but in conjunction with others may be quite valid. All of them should be carefully noted in the field since they are not so easily determined from dry material.

The following is Sir David Prain's classification. Comment is unnecessary where no alteration is proposed.

Section Eumeconopsis. Plants with simple hairs, setae, or prickles.

I. Anomalac.

II. Cambricac.

III. Cumminsia. Defined as perennials with variously cut sparsely hirsute leaves; 4-petalled purple or blue flowers; and a long slender capsule in which dehiscence is not confined to the apex.

The species included here are: *M. lyrata*, *M. compta*, *M. polygonoides*, and *M. betonicifolia*.

*M. lyrata* (originally *Cathcartia lyrata*) is a rare Sikkim plant with pale blue flowers. *M. compta* is a Chinese plant with purplish flowers. I have compared the two, and can see no difference except in the incision of the leaves, a distinction which is never very constant in *Meconopsis*.

Mr. E. H. Evans of Edinburgh agrees that *M. compta* = *M. lyrata*. Moreover, Farrer found a *Meconopsis* on the north-east frontier of Burma (lat. 26° 0' N., long. 98° 40' E.) which has been referred to the Sikkim *M. lyrata*. As this was found not far from where *M. compta* was found the latter is probably also *M. lyrata*.

To these must be added the yellow-flowered *M. florindae*, K. W. 6038, found in Tibet. This species in habit, foliage, capsule, and root is clearly allied to *M. lyrata*; but it has 5-7 petals. In some specimens the leaf is entire, narrow-linear, in others pinnatisect.

<sup>1</sup> Kew Bulletin, 1915.

*M. lyrata* is said to be perennial, though there is nothing in the small radish-like root to suggest it; nor did I see signs of it in this yellow-flowered species.

*M. lyrata* is a high alpine, but K. W. 6038, from Kongbo (lat.  $29^{\circ} 45'$  N. long.,  $94^{\circ} 45'$  E.), is a forest plant from 11,000 ft. We did, however, find in fruit on the eastern Himalaya, a little south of the above point, an alpine species of this aggregate—probably *M. lyrata* again.

*N. betonicifolia*, the only certain perennial included in Cumminsia, has barbellate hairs and leaves like *M. superba*. It must therefore be removed to the section Polychaetia. It differs from *M. Baileyi* in not having the base of the stem leaves auricled, in its longer style, and in its almost glabrous capsule.

Our amended group, Cumminsia, would thus comprise: *M. lyrata*, *M. polygonoides*, and *M. Florindae*. They are slender biennial (annual?), almost glabrous herbs, not exceeding a foot, with narrow stem leaves, deeply incised or almost entire; blue, purple, or yellow flowers; a small radish-like root, a long narrow fusiform capsule, dehiscing to half its length or more; and short style.

The discovery of *M. Florindae* has added complications and widened the definition; on the other hand the removal of *M. betonicifolia* has drawn the remaining species closer together.

#### IV. Decorae.

V. Aculeatae. There are seven known species, three Indian, three Chinese, and one widely distributed Tibetan. The Indian species are *M. aculeata*, *M. latifolia*, and *M. sinuata*, and they all have four petals. The Chinese species are *M. Prattii*, *M. rudis*, and *M. speciosa*, and they all have more than four petals. The Tibetan plant is *M. horridula*, which also has more than four petals. To this we must now add two more species collected in eastern Tibet in 1924, *M. Prainiana* (K. W. 5909) and *M. Cawdoriana* (K. W. 5751). The former has 4, casually 5 petals, and is thus related to the Indian group. It is separated from *M. aculeata* and *M. latifolia* by its entire leaves, though it has the green stigma of the former; and from *M. sinuata*, both by its leaves—narrow-linear with obtuse instead of acute apex, and margin not sinuate—and still more by its capsule, obovoid, with very long style, instead of obconic.

The latter has 6 petals, and in habit resembles *M. horridula*, from which it differs in its capsule as well as in minor details. Thus we now have three Indian, three Chinese, and three Tibetan species of Aculeatae.

#### VI. Primulinae.

VII. Bellae. The species are *M. bella*, *M. impedita*, *M. concinna*, *M. venusta*, and *M. Delavayi*. Sir David Prain includes *M. Baileyi*, but that has barbellate hairs, and resembles *M. betonicifolia* in many respects.

It is therefore necessary to remove it also to section Polychaetia.

I am satisfied that a plant we collected in Kongbo (K. W. 5784) is *M. Baileyi*, and of its relationship there can be no doubt ; it has nothing to do with the Primulinac-Bellae group.

*M. Baileyi* was incompletely described by Sir David Prain from a fragment collected by Major F. M. Bailey during his journey in Tibet in 1913. The material was very slight—one or two flowers without foliage, fruit, or stem, and no record of habit ; it is therefore not surprising that Sir David was led astray, since there was practically no clue to its affinity other than the 4 petals. I have examined the specimen in Kew Herbarium. The hairs of the stem are not barbellate, and only occasionally can one detect a barbellate hair on the young ovary. It is the same with our own specimens ; the leaf-hairs are barbellate, the stem and flower hairs are simple or only slightly barbellate. The note accompanying Bailey's specimens says it was collected in flower on July 10, 1913, at Lunang (lat. 29° 45' N., long. 94° 45' E.) ; our specimens were collected at precisely the same spot at the same time, and there is no other species of *Meconopsis* in the neighbourhood with which it could be confused.

The following is the amended description from the living plant :

*Meconopsis Baileyi*, Prain, amended Ward. Rootstock perennial, sending up annual stems from 2½ to 3½ ft. high. Stem with scattered simple hairs. Radical leaves and lowest (one or two) stem leaves petiolate. Lamina 7–9 in. long, 3 in. wide, narrow oval with acute apex and sometimes asymmetrical base, more or less decurrent ; broadly crenate, with stiff barbellate hairs scattered along the margin ; petiole 5–6 in. long (or sometimes much longer) expanded at the base. Upper leaf surface dark green, with stiff scattered hairs ; midrib colourless, prominent ; lower surface paler with prominent venation, glabrous except for scattered hairs confined to the midrib. Stem leaves similar but with auricled base ; sessile, lanceolate, both surfaces with scattered hairs ; the lowest as much as 12 in. long and 3 in. wide.

Buds ovoid, densely hairy.

Flowers solitary, axillary (except the terminal one) in an irregular cyme, borne on scapes 6–8 in. long, nodding ; the uppermost two reaching almost to a level with the terminal one. Flowers 4 in. in diameter. Sepals 2, oval, hairy outside. Petals 4 (rarely 5–6), sky blue with darker veins, almost circular, 2 in. in diameter. Stamens numerous, multiseriate ; filaments colourless, ⅜ in. long ; anthers golden yellow. Ovary ovoid, completely covered with silken, silvery, usually simple hairs. Style very short, ⅙ in. long, fluted ; stigma pale green, star-shaped, with 4–6 flattened rays which curve down over the fluted style ; top flattened.

Capsule 1½ in. long obovoid, opening ⅓ of its length by 4–6 valves.

Polychaetia. Plants more or less covered with barbellate hairs.

VIII. *Grandes*. In this aggregate Sir David Prain includes *M. integrifolia*, *M. pseudo-integrifolia*, *M. grandis*, *M. simplicifolia*, *M. quintuplinervia*, and *M. punicea*.

The yellow-flowered species have given rise to some confusion which I will attempt to clear up. For many of the facts stated below I am indebted to Sir David himself.

*M. integrifolia*. This was the original species discovered in north-east Tibet by the Russians, and named *Cathcartia integrifolia* by Maximowicz. Franchet renamed it *Meconopsis integrifolia*. There is no style, but there are 4 to 6 vertically radiating stigmatic rays. The species is widely distributed in Chinese Tibet, in meadows and on open *Rhododendron* moorland; but we did not meet with it in the Tsangpo valley.

The second yellow-flowered species to be discovered in western China, this time by the French missionaries, resembled *M. integrifolia*, but had a very short style with flattened stigma. However, Franchet, supposing it to be identical with Maximowicz's plant, called it also *M. integrifolia*, having in the meantime transferred Maximowicz's plant from *Cathcartia* to *Meconopsis*.

The third species discovered in north-east Tibet, again by the Russians, was raised from seed sent to this country by the St. Petersburg Botanic Gardens (as they were then). It was assumed to be *M. integrifolia*, and was so called.

Thus there were, as we now know, three plants actually distinct masquerading under the one name. Workers were now beginning to compare the three gatherings.

In 'Flora and Sylva', vol. iii, p. 80, Mr. A. K. Bulley mentions *M. integrifolia*. Later (p. 191) he points out that there are *two* plants confused under the one name, though he seizes on an unessential distinction to prove it. The fact is, though he is speaking of one plant (*M. integrifolia*) he is all the time describing another (*M. pseudo-integrifolia*), as can be seen from the figure.

Mr. Bulley raised this plant from Koslov's seed, and says that it bore only basal 1-flowered scapes, whereas the plant hitherto known as *M. integrifolia* had a central branching stem. This distinction is not valid, however.

It was Sir David Prain who, on seeing the plant raised from Koslov's seed, recognized it as distinct from the original Russian plant (*M. integrifolia* of Franchet) by its long style, and described it under the name *M. pseudo-integrifolia*.

We now have (i) the original plant discovered by the Russians, *M. integrifolia*, Franchet; (ii) the plant found by the French missionaries, also referred to *M. integrifolia*; (iii) the plant discovered by the Koslov

Expedition, distributed as *M. integrifolia* and subsequently described as *M. pseudo-integrifolia*, Prain. Now was the French missionaries' plant really *M. integrifolia*? About this time Mr. E. H. Wilson collected true *M. integrifolia* in Szechuan. Subsequently Mr. G. Forrest sent home a yellow-flowered poppy which he said was not *M. integrifolia*, or at all events not Mr. Wilson's *M. integrifolia*, and was therefore probably a new species. Sir David Prain then went into the matter once more, and found that Forrest was right; his plant was not true *M. integrifolia*; but it *was* the same as the French missionaries' plant, called *M. integrifolia* by Franchet. Clearly, then, Franchet had confused another species or variety, besides *M. pseudo-integrifolia*, with *M. integrifolia*.

Sir David Prain, though not yet prepared to accord specific rank to the missionaries' plant, suggested to Professor Bayley Balfour that it might be called either *M. integrifolia*, var. *microstigma*, or *M. pseudo-integrifolia*, var. *brevistyla*, according to which view one took of it. Professor Balfour, however, decided to split the difference, and the plant was issued as *M. integrifolia*, var. *brevistyla*.

Finally, there is *M. integrifolia*, var. *Souliei* of Fedde, which, according to Sir David Prain, is simply *M. pseudo-integrifolia*. The only question that arises now is whether *M. integrifolia*, var. *brevistyla* is worthy of specific rank or not.

I have frequently seen and collected all three varieties in Yunnan and Szechuan, and they appear to me to be easily distinguishable in the field. Nor is the distinction confined to the style and stigma, though doubtless most easily observed there in herbarium specimens. Variety *brevistyla*, therefore, has just as good a claim to specific rank as has *M. pseudo-integrifolia*, and it would be simpler and in accordance with facts to call it *M. brevistyla*.

The three yellow-flowered Grandes, then, may be distinguished as follows:

*M. integrifolia*. Hairy plant of 12–15 in. There is a central stem, bearing a number of 1-flowered axillary pedicels. Flowers gamboge, often very large and globe-like. Capsule ovoid, with 4–6 ridge-like stigmatic rays. Style 0. Dehiscence by 4–6 apical ports. In open peaty moorland, 13,000–14,000 ft., scattered in small clusters. In cultivation, and does fairly well.

*M. pseudo-integrifolia*. Plant of 30 in., not so hairy as the last. There is a well-developed central stem, bearing axillary 1-flowered pedicels which eventually all appear to spring from one level, but do not grow as tall as the main scape. Flowers sulphur, paler than in the last, not so large, flatter, and more open. Capsule obconic, nearly glabrous. Style about a quarter the length of the ovary, with fluted, knob-like stigma. Capsule

opening by 8–10 valves, the tips of the valves curling back. Scattered in small colonies in shady situations, sometimes in forest or under trees along the banks of streams. In cultivation, usually under the name of *M. integrifolia*, and does fairly well.

*M. brevistyla*. Plant of 12 in. or less, bearing one or two single-flowered pedicels arising at or near the base of a central stem. Whole plant very hairy, with long champagne-coloured hairs. Flowers cadmium-yellow, often very large. Capsule narrow ovoid. Stigma flattened into a disc-like structure, consisting of 6–8 stellate rays; style very short or 0. The capsule opens by 6–8 small apical ports. Plants widely scattered on barren limestone or igneous screes above 16,000 ft.—the last flowering plants met with. Not in cultivation.

The reason why confusion has arisen in gardens is now obvious. There are three names abroad, but only two plants. *M. brevistyla* is not in cultivation and probably never will be in this country. It is a sub-arctic species. Plants called *M. brevistyla*, or var. *brevistyla*, generally turn out to be *M. integrifolia*. As to the point raised in 'Flora and Sylva' by Mr. Bulley that *M. integrifolia* (meaning in this case *M. pseudo-integrifolia*) has no central stem: it frequently happens that this species, coming up late after a severe winter, is checked and stunted. The axillary pedicels, which, as stated, sometimes spring from one level, may appear to rise directly from the ground, owing to the non-development of the central stem; but the central stem is there under the surface, and may develop later. The habit is of no diagnostic value, and may easily be induced artificially.

I pass on now to the Tibetan yellow-flowered species.

K. W. 5749 has the habit and style of *M. pseudo-integrifolia*, to which species it comes nearest; but the style is much longer in proportion to the ovary ( $\frac{1}{3}$  to  $\frac{1}{2}$  the length), which is obovoid, very hairy, and opens by 6 valves; stigma depressed with divaricate rays. The leaves are linear instead of linear-lanceolate, and the whole plant is more hairy than *M. pseudo-integrifolia*. These characters may be sufficiently distinct to raise the plant to specific rank.

K. W. 5984, 5910. This species comes nearest *M. brevistyla*. There is no style and the stigma is hollow, receptive only on the inside, opening after the flower opens, with six diverging decurrent rays.

The five species enumerated above form a compact aggregate within the group *Grandes*, characterized by yellow flowers borne on a central stem. It would, I think, be logical and convenient to regard them as an aggregate comparable to the other groups; the name *Integrifoliae* suggests itself.

*Grandes* as amended would then comprise: *M. simplicifolia*, *M. quintuplinervia*, *M. grandis*, and *M. punicea*. They are plants with blue or purple solitary nodding flowers borne on distinct basal scapes. The only species

about which it is necessary to say anything is *M. simplicifolia*. Some confusion seems to have arisen in gardens concerning this species. It is frequently said that *M. simplicifolia* has small purple or dark-blue flowers and is perennial. In 1913 Bailey sent home seed of a *Meconopsis* collected in the eastern Himalaya, and plants raised at Edinburgh flowered in 1915. They had sky-blue flowers, and according to Sir David Prain agreed in all respects with *M. simplicifolia* as known from Sikkim, Nepal, and Tibet. This form, however, which is biennial, became known as *M. simplicifolia*, var. *Baileyi*. It is clear, however, from what Sir David said and from the figure in the 'Botanical Magazine' (tab. 8364) that the original *M. simplicifolia* of Wallich was a biennial with sky-blue flowers. The purple or dark-blue flowered perennial may be an inferior, or possibly a different species. Is it by any chance *M. Henrici*?

Nothing is said about scent in any of the above plants. In Tibet we collected north of the Tsangpo a plant, K. W. 5737, which agreed with *M. simplicifolia*, var. *Baileyi* in all respects, but it is also very fragrant. South of the Tsangpo, on the Assam Himalaya, north flank, we collected a purple-flowered plant, K. W. 5855, which agreed closely with K. W. 5737, but it had a slightly longer style and no fragrance, or a faint odour of Shirley Poppy. On the south flank of the same range we collected, in fruit only, a similar plant, K. W. 6245; this had a still longer style and differed again in producing twice as many scapes as the former species—6 to 8 as against 3 to 4. Sir David Prain suggests that *M. simplicifolia* is a Tibetan plant which has crossed the passes southwards; and its considerable extension towards the north and east (lat. 29° 45' N., long. 94° 45' E.) supports that view.

#### IX. Torquatae.

X. Robustae. *M. superba*, *M. paniculata*, *M. robusta*, *M. napaulensis*, *M. Wallichii*.

I have seen only an imperfect herbarium specimen of *M. superba*, but there is no mistaking its affinity with *M. betonicifolia* and *M. Baileyi*. It seems to me advisable to remove it from the aggregate Robustae and to make these three species the nucleus of a new group, Superbae.

If now we remove *M. superba*—and it has been proved that *M. napaulensis* = *M. Wallichii*—the Robustae, amended, comprise three species, viz.: *M. paniculata*, *M. robusta*, *M. Wallichii*.

A slight correction of Sir David Prain's account of *M. Wallichii* is necessary. He says that I met with typical *M. Wallichii* (i. e. with sky-blue flowers) in Upper Burma in 1914—without precise locality. As a matter of fact it was the variety *fusco-purpurea* (the so-called *M. napaulensis*) that I collected both in 1914 and again in 1919, when Farrer also found it in the same district, namely, near Hpimaw, lat. 26° 0' N., long. 68° 40' E.

XI. *Chelidonifoliae*.

The alterations suggested in the present classification of *Meconopsis* may now be briefly summarized thus :

(i) Removal of *M. bctonicifolia* from Cumminsia and re-definition of that group.

(ii) Removal of *M. Baileyi* from Bellae.

(iii) Removal of *M. superba* from Robustae.

(iv) Recasting of these three species into a new group—Superbae.

(v) Division of the group Grandes into two—a yellow-flowered, compound inflorescence group (*Integrifoliae*) and a blue-purple, simple inflorescence group (*Grandes*).

Thus two new groups are suggested, making thirteen in all. It is also proposed to sink *M. compta* in *M. lyrata*, while three new species are added. It may be necessary to make three or more new species later when the varieties of *M. simplicifolia*, *M. pseudo-integrifolia*, and *M. brevistyla* come to be examined critically.

Seed of nearly all these Tibetan species has germinated in this country.

## EXPLANATION OF PLATE XVI.

Illustrating Mr. F. Kingdon Ward's paper on the genus *Meconopsis*.

Fig. 1. *Meconopsis Prainiana*, Ward (K. W. 5909), growing among dwarf Rhododendron. Nam La, Tibet, 14,000 ft.

Fig. 2. (K. W. 5766) *M. simplicifolia*, var. ?, among dwarf Rhododendron. Nyima La, Tibet, 14,000 ft.

Fig. 3. (K. W. 5784) *M. Baileyi*, Prain. Lunang, Tibet, 11,000 ft.

Fig. 4. (K. W. 5749) *M. pseudo-integrifolia*, var. ?, among dwarf Rhododendron. Temo La, Tibet, 14,000 ft. (Photograph by Lord Cawdor.)





1.



2.



4.



3.

With col.



## Further Investigations of the Chemical Nature of the Cell-membrane.

BY

F. M. WOOD, B.Sc., PH.D., F.I.C.

With three Figures in the Text.

IN continuing the study of the chemical nature of the cellulose membrane, the first results of which have already appeared (3), it was found necessary to investigate the occurrence of protein in the cell-wall. As one of the objects of the research was to distinguish definitely between pectin and cellulose in the cell-wall, the effect of other substances present upon the reagents used could not be ignored.

R. M. Tupper-Carey and J. H. Priestley (1) suggest that protein closely linked to the cellulose is most probably the substance which prevents the reaction with iodine and sulphuric acid, and that it is generally a constituent of the cell-wall of plumule, radicle, and root under normal conditions, whereas in the adult parenchyma of root and green shoot it does not occur (p. 128).

It must be pointed out that these writers base their observations on experiments conducted chiefly with *Vicia Faba*, although some experiments were made with *Phaseolus multiflorus* (p. 110). Thus their investigation of the protein contents of the cell-wall was confined to plants whose cell-contents are rich in protein. By the application of a test for protein which is used in technical work, the present author has developed a new method for the detection of protein in the cell-wall.

The reaction depends upon the setting free of iodine from potassium iodide in the 'chloramine' reaction. The protein-containing material is submitted to the action of chlorine, when a chloramine is produced. It is then washed with distilled water and treated with sodium hydrogen phosphate to convert traces of iron into the phosphate. On further washing and adding potassium iodide solution, iodine is set free where protein compounds are present.

The test was carried out in the following way: living stems and roots of many plants were used, and sections were cut, generally transversely, by means of a hand or sliding microtome according to the character of the material.

At first the sections were treated with freshly made chlorine-water for

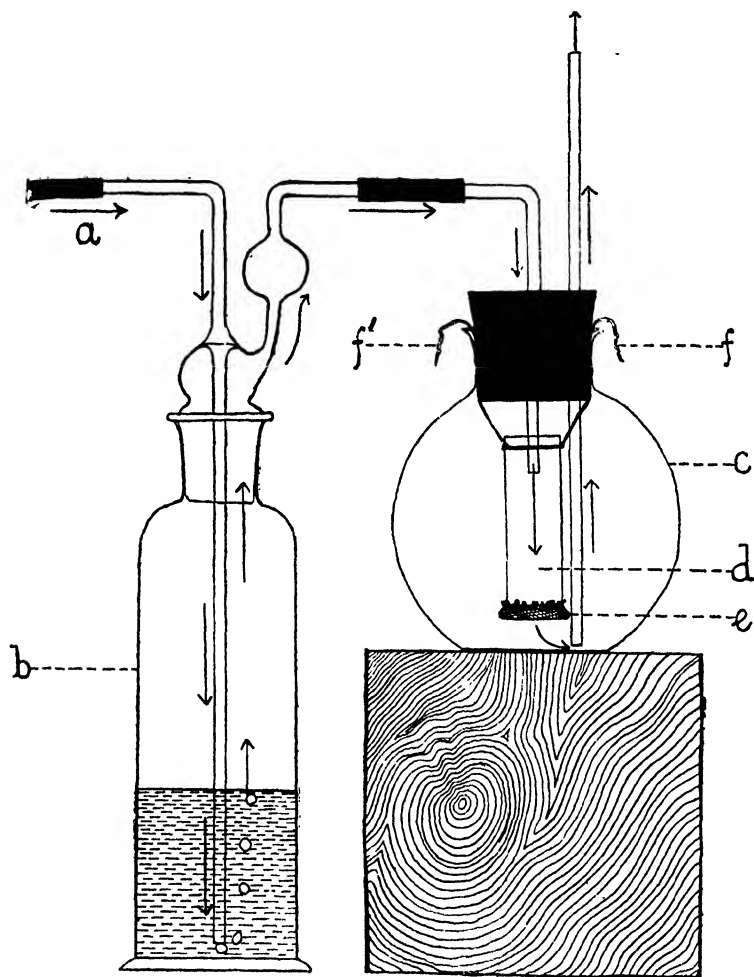


FIG. 1. First apparatus used in chlorination. *a.* Arrows show the passage of the chlorine gas from the gas-cylinder. *b.* Wash-bottle containing water to free chlorine from hydrochloric acid. *c.* Wide-mouthed flask in which sections were chlorinated. *d.* Tube containing sections. *e.* Muslin upon which sections were laid. *f, f'.* Strings supporting tube.

periods varying from 2 hours to 4 weeks; washed in water, soaked in sodium hydrogen phosphate solution, again washed, and treated with a freshly made solution of potassium iodide. This method did not give sufficiently definite results even after prolonged periods of exposure, and chlorine gas was accordingly used.

By means of the apparatus shown in Fig. 1, the sections were exposed to the action of wet chlorine from a gas-cylinder either at ordinary temperature or at  $45^{\circ}$ – $50^{\circ}$  C., for periods varying from  $\frac{1}{2}$  hour to  $1\frac{1}{4}$  hours. They

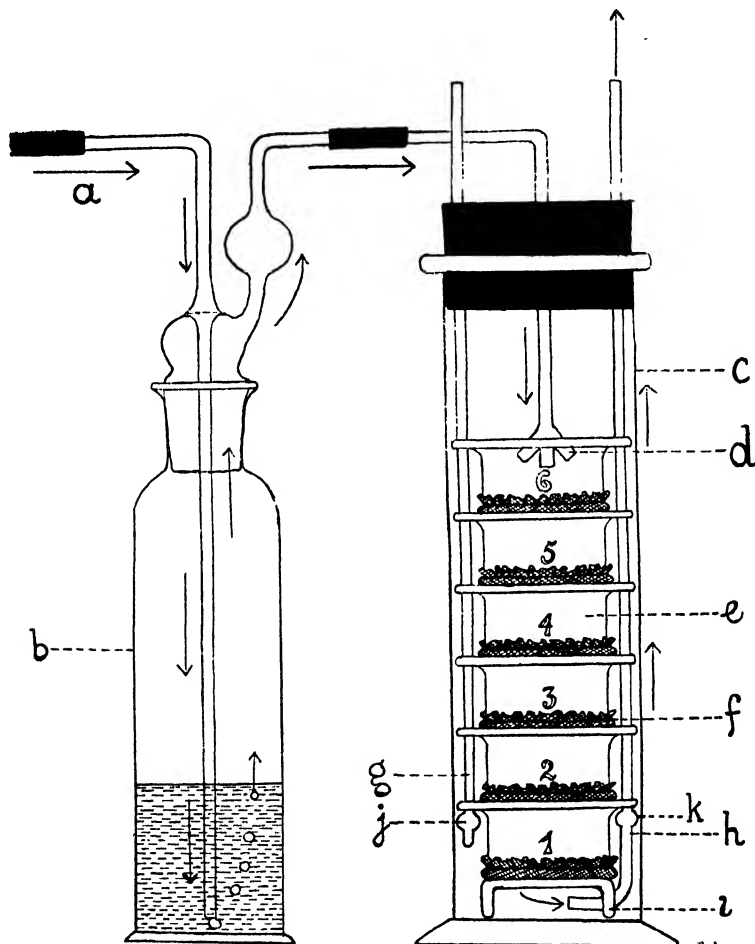


FIG. 2. Improved apparatus used in chlorination. *a*. Arrows show the passage of the chlorine gas from the gas-cylinder. *b*. Wash-bottle containing water to free chlorine from hydrochloric acid. *c*. Glass jar containing sections. *d*. Four-way tube through which the chlorine enters. *e*. Battery of dishes in which sections are exposed to chlorine gas. *f*. Muslin upon which sections lie. *g*. Supporting rod holding battery of dishes. *h*. The other supporting rod, which is also a tube through which the chlorine is drawn off. *i*. Glass stand upon which the battery of dishes rests. *j*. Glass button. *k*. Glass button.

were placed on muslin stretched across a small tube open at both ends, and the gas passed directly through them.

As the work proceeded a more convenient apparatus was devised (Fig. 2). This consisted of a tall glass jar within which a 'battery' of small glass dishes were arranged. These were fitted with removable muslin

bottoms as before and were provided with glass handles on either side, and arranged one above the other, being held together by straight glass tubes passing through the handles. The tube *h* formed the outlet for the gas, *g* being merely a support. Glass buttons at *j* and *k* prevented the dishes slipping off the tubes. The two tubes passed through a large rubber stopper which closed the mouth of the jar. The whole 'battery' rested upon a glass stand *i*, so that the gas could pass freely through the last dish. This also lifted the dish out of the water which always accumulates at the bottom of the jar during chlorination. By this arrangement all the dishes were removed easily at one time.

The chlorine entered the apparatus by tube *d*, which had a four-way entrance on the side within the cork to provide for better distribution of the gas. Each dish was permanently numbered, so that it was a simple matter

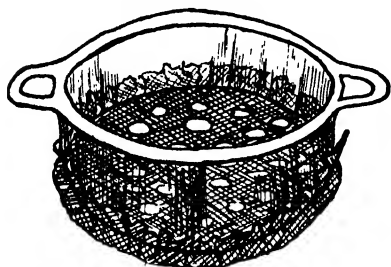


FIG. 3. A single dish showing the sections laid upon the muslin bottom, and the two handles through which the supporting rods pass.

to expose six different kinds of sections at one time without risk of mixing them. The apparatus effected a great saving both in time and chlorine gas.

Before coming into contact with the sections the gas was washed with water to remove hydrochloric acid, which might produce hydrocellulose in the cell-walls, and possibly help to disintegrate the tissues. Raising the temperature to 40°–50° C. quickened the reaction, but it was considered

safer to dispense with this heating, as it introduces an additional factor.

Sections of herbaceous stems do not require such a long chlorination as woody ones;  $\frac{3}{4}$  hour is sufficient for material of this type. The maximum time necessary for any section was  $1\frac{1}{2}$  hours.

Excessive chlorination may render the section too 'tender' for further convenient manipulation, so care must be taken that the minimum time is allowed, especially when the material is of a succulent nature. At the end of the reaction the excess of chlorine was removed by pumping air through the apparatus and then washing the sections on the muslin in a stream of distilled water.

Various strengths of sodium hydrogen phosphate and potassium iodide were tried. For the former a 10 per cent. solution gave the best results, while for the latter a 5 per cent. solution was found useful.

The iodide solution must be freshly prepared at the time at which the micro-examination is made, as traces of iodine are set free if the solution is kept ready-made, and this would vitiate the results.

The strength of the potassium iodide solution is not of vital importance, as only a definite amount of iodine is set free, and no more, however

strong the solution may be; the 5 per cent. solution was found to be suitable.

The sections were mounted in potassium iodide and examined under the microscope immediately, the source of illumination being a 'Fullolite' electric bulb with a neutral colour screen. Where protein is present a yellow colour is produced due to liberated iodine. If starch is present the iodine set free by the cell-contents stains this blue. Lignified cell-walls become either dark-brown or pink according to their constitution. Cell-walls composed of cellulose, oxycellulose, or hydrocellulose are uncoloured.

After 20 to 30 minutes the iodine set free by the cell-contents tends to wash into the uncoloured cell-walls, so that the examination must be made at once. However, there is ample time for observing and recording the results.

A number of sections of the same specimen is chlorinated so that should time prove insufficient for a critical examination of the first selected, others similar are in readiness for the final examination. When very little protein is set free, it is of advantage to wash the section in water and to mount it in a very dilute solution of soluble starch, which must be freshly made. Wherever iodine is set free a blue colour is produced. This has been found of value when it is difficult to perceive whether there is any yellow colour or not, and it also affords a means of differentiating between the yellow or brown colour due to the liberated iodine and any similar colour produced by chlorine with lignin and suberin. After chlorination the sections are somewhat opaque, but on immersion in sodium hydrogen phosphate solution they at once become transparent.

Where mucilaginous or resinous secretions occur, the material is treated for a short time with 60 per cent. alcohol to remove them, as otherwise the chlorine cannot penetrate properly. In the majority of cases this was found unnecessary. The test was found to be unaffected by the treatment of the sections with alcohol, but in order that the conditions should be as uniform as possible in all cases, the alcohol-treated material was washed in water before chlorination.

Somewhat to the author's surprise the chlorinated sections could be kept for weeks in distilled water without suffering disintegration, and uniform results were obtained with sections examined immediately after chlorination and others of the same kind after four weeks' storage in distilled water. Possibly there was sufficient chlorine remaining in the material to preserve it from decay. Temperature was a factor in the preservation of the sections in this way, as directly the temperature of the air rose to about 15°-18° C. moulds developed and began to destroy the tissue. Over-chlorinated sections, which had become too 'tender', remained intact while floating in water, and it was only the pressure of the cover-slip that crushed and disintegrated them. As far as an examination could take place without a cover-slip, they were still fit for examination.

It will be seen that an endeavour was made to limit the reagents involved in the reaction, and to this end, except for the occasional treatment with alcohol, nothing was used except chlorine, sodium hydrogen phosphate, potassium iodide, and occasionally soluble starch. The idea was to determine, as nearly as possible, the condition in life of the constituents of the cell-wall.

That effective chlorination had taken place was proved by submitting some of the sections both before and after chlorination to Knaggs's test for oxycellulose (4) and comparing the results. In all cases this showed that oxycellulose had been produced by the chlorine.

The results obtained before chlorination provide further information on the occurrence of oxycellulose in the cell-wall of various plants, and many of these results are given in Table I. In this test, as described by the present author in a previous paper (3), the sections are washed in dilute hydrochloric acid, then with water dyed deeply with benzopurpurin, and washed again with acid, when a blue colour is produced. They are then washed in water, when that part of the cell-wall which is not oxidized becomes red, but the oxidized part remains blue-black in colour, the whole having a bluish red appearance. The persistence of this colour on washing instead of the reappearance of clear red shows that oxycellulose is present. It is somewhat curious that in the final washing tap-water gave more reliable results than distilled water. Occasionally distilled water fails to remove the blue colour, even when oxycellulose is known to be absent, but this never happens when ordinary London tap-water is used.

A large number of plants were examined by the chloramine test. These included leaf, stem, and root structures, young and old, and both Monocotyledons and Dicotyledons. Some of the results obtained with these specimens are given in Table II.

TABLE I. *Oxycellulose by Knaggs's Test.*

The presence of oxycellulose is shown by the sign +, its absence by —.

A. LEAVES.

<i>Plant.</i>	<i>Before chlorination.</i>	<i>After chlorination.</i>
<i>Orchis</i> sp. . . . . (epidermis only)	—	+
<i>Iris foetidissima</i> . . . .	—	Slightly +
<i>Iris xiphila</i> . . . .	—; but cuticle, sclerenchyma, and xylem uncoloured	+; sclerenchyma only slightly yellow
<i>Arum maculatum</i> . . . .	—; sclerenchyma +	Slightly +; sclerenchyma strongly +



TABLE I (continued).

## B. PETIOLES.

Plant.	Before chlorination.	After chlorination.
<i>Caucalis anthriscus</i> . . .	Slightly +; sclerenchyma distinctly +; hairs and phloem —	+; but hairs and sclerenchyma yellow
<i>Galanthus nivalis</i> . . .	—; xylem very slightly +	+
<i>Arum maculatum</i> . . .	—; but sclerenchyma +	Slightly +; sclerenchyma strongly +
<i>Anemone nemorosa</i> . . .	+; xylem only slightly +; outer layers of cortex and epidermis —	+

## C. AERIAL STEMS.

<i>Vicia Faba</i> . . . . .	—; slightly + in xylem and epidermis	+; but only very slightly in cortex
<i>Phaseolus multiflorus</i> . . .	—	Very strongly +
<i>Muthiola incana</i> . . . . .	—	+; but xylem and sclerenchyma yellowish pink
<i>Caucalis anthriscus</i> . . . . .	—; + in pith and patches of sclerenchyma	+; hairs and sclerenchyma only yellow
<i>Cheiranthus Cheiri</i> . . . . . (young stem)	Very little colour anywhere except in epidermis, generally —, but hairs, sclerenchyma, and xylem +	Slightly +
<i>Cheiranthus Cheiri</i> . . . . . (old stem)	—; but hairs, xylem, and sclerenchyma +	Slightly +
<i>Saxifraga umbrosa</i> . . . . .	Slightly +; but pith —	+
<i>Helleborus viridis</i> . . . . .	— phloem and cortex; very slightly + xylem and pith; epidermis and collenchyma brown	+; some of the xylem is yellow; protoxylem strongly +
<i>Lamium purpureum</i> . . . . .	—; but epidermis and cortex brownish red	+; xylem —
<i>Euphorbia amygdaloides</i> . . .	—; but only slightly + in xylem	Strongly +; xylem slightly +
<i>Ligustrum vulgare</i> . . . . .	—; but + in older xylem and cuticle	+; strongly + in old xylem, cuticle, and cork
<i>Ulmus campestris</i> . . . . .	—	+
<i>Prunus amygdalus</i> . . . . . (double)	Slightly +; pith cells near protoxylem —; xylem and sclerenchyma uncoloured	Slightly +; xylem bluish-brown; sclerenchyma slightly yellow
<i>Prunus amygdalus</i> . . . . . (single)	— cortex; + phloem, cork, pith; xylem yellow; sclerenchyma uncoloured	+ cortex, phloem, and protoxylem; cork, sclerenchyma, xylem, pith all yellow
<i>Fagus sylvatica</i> . . . . .	— very slightly xylem and phloem; pith slightly +; cork brown; sclerenchyma uncoloured	+; xylem and cork yellow
<i>Populus balsamifera</i> . . . . .	—; xylem and sclerenchyma uncoloured; cork yellow; cortex sometimes slightly +	+; sclerenchyma and xylem yellow
<i>Salix alba</i> . . . . . (young stem)	—; but pith, cork, and sclerenchyma yellow	+
<i>Salix alba</i> . . . . . (old stem)	—; but pith +; cork and sclerenchyma yellow	+; but sclerenchyma, xylem, and pith yellow
<i>Prunus communis</i> . . . . .	—; but pith very slightly +; xylem uncoloured	+; sclerenchyma, xylem, and pith yellowish
<i>Corylus Avellana</i> . . . . .	—; cork yellow	+; cork and xylem yellow

TABLE I (*continued*).C. AERIAL STEMS (*continued*).

<i>Plant.</i>	<i>Before chlorination.</i>	<i>After chlorination.</i>
<i>Skinnera japonica</i> . . .	—; xylem yellow in parts, i e. those cells which become red in the chloramine test (see Table II), i.e. younger xylem and protoxylem	+; xylem staining red in chloramine test is yellow; outer layers of cortex yellow
<i>Bauera rubioides</i> . . .	—; little colour in xylem; slightly + phloem and medullary rays	+, except xylem, which is yellow
<i>Ribes nigrum</i> . . .	Little colour absorbed; slightly + protoxylem and pith; xylem —; cortex, phloem, brown; cork yellow	+; but xylem and cork yellow, and protoxylem strongly +
<i>Centradenia floribunda</i> .	—	+; secondary xylem more strongly + than protoxylem; cork brown
<i>Caltha palustris</i> . . .	Slightly +; but pith —	Strongly +
<i>Lamium album</i> . . .	—; but + in epidermis, cortex, and xylem	Slightly +
<i>Rumex conglomeratus</i> . .	+; but cortex — and pith only coloured yellowish brown	Strongly +
<i>Cytisus scoparius</i> . . .	Slightly +; but sclerenchyma and parts of xylem only yellow	+
<i>Brassica oleracea</i> . . .	—; but stele and sclerenchyma only yellow	+; but sclerenchyma —, and parts of xylem only yellow
<i>Acer Pseudo-platanus</i> . .	—; but cork uncoloured and pith very slightly +	+; but pith —
<i>Carpinus Betulus</i> . . .	Little colour absorbed; — xylem and phloem; pith +; cortex and cork only yellow	--
<i>Syringa vulgaris</i> . . .	—; older part of secondary xylem yellow; pith slightly —	Slightly +

## D. UNDERGROUND STEMS.

<i>Bellis perennis</i> . . .	Slightly +	Strongly +
<i>Viola canina</i> . . .	— cortex and most of xylem; + xylem vessels; slightly + phloem; sclerenchyma uncoloured	+ strongly, except xylem, which is yellow
<i>Fragaria vesca</i> . . .	—	+; xylem yellow
<i>Primula veris</i> . . .	—; slightly + in cortex and pith	Very strongly +; but epidermal layer —
<i>Anemone nemorosa</i> . .	Slightly +	+

## E. ROOTS.

<i>Saxifraga umbrosa</i> . .	+ <sup>4</sup>	+
<i>Vicia Faba</i> . . .	—; + in xylem and piliferous layer	+; only slightly + in cortex
<i>Phaseolus multiflorus</i> .	—	Strongly +
<i>Euphorbia amygdaloides</i> .	—; slightly + in xylem	Strongly +; xylem slightly +
<i>Helleborus viridis</i> . .	— phloem; outer layers slightly +; inner layers of cortex —; xylem uncoloured; piliferous layer dark brown	+; but piliferous layer brown and xylem yellow
<i>Ranunculus Ficaria</i> . .	—; cortex very slightly +	+, except piliferous layer and cells just below this

TABLE II.  
Results obtained by the Chloramine Test on Plant Tissues.

A. LEAVES.

Plants . . . .	<i>Orchis</i> sp.	<i>Iris foetidissima</i> .	<i>Iris xiphila</i> .	<i>Arum maculatum</i> .
Epidermis . . . .	No colour; contents of stomata dark brown.	No colour; contents distinctly coloured.	No colour; contents of stomata deep blue.	No colour.
Cortex . . . .	—	—	No colour.	No colour; layer of cells above each bundle dark brown.
Pith . . . .	—	—	No colour.	No colour.
Sclerenchyma: a. Cortical. . . . b. Stelar . . . .	— —	— —	— Dark brown.	No colour. —
Xylem: a. Primary . . . . b. Secondary . . . .	— —	— —	Dark brown. —	Brown. —
Phloem . . . .	—	—	No colour.	No colour.
Remarks . . . .	—	—	—	—

TABLE II (continued).

B. PETIOLES.				
	<i>Caucalis anthriscus.</i>	<i>Gadanthus nivalis.</i>	<i>Arum maculatum.</i>	<i>Aueunone nemorosa.</i>
Plants . . . .		—	—	—
Hairs . . . .	No colour; contents yellow.			
Epidermis . . . .	Slightly yellow.	No colour.	No colour.	No colour.
Cortex . . . .	No colour; layer of cells above each bundle had blue contents.	No colour.	No colour; layer of cells above each bundle had deep brown contents.	No colour.
Pith . . . .	No colour.	No colour.	No colour.	No colour (collenchyma).
Sclerenchyma :				
<i>a.</i> Cortical . . . .	No colour.	No colour.	No colour.	Pale pink.
<i>b.</i> Stelar . . . .	—	—	—	—
Endodermis . . . .	—	—	—	—
Xylem :				
<i>a.</i> Primary . . . .	Slightly yellow.	No colour.	Brown.	Dark brown, almost black.
<i>b.</i> Secondary . . . .	—	—	—	—
Phloem . . . .	No colour.	No colour.	No colour.	No colour.
Remarks . . . .	Sections were very 'tender'. The first two epidermal layers came away from the rest very easily.	The sections were difficult to examine as the cell-contents set free a large amount of iodine.	Sections were somewhat 'tender'.	—

C. AERIAL STEMS.

Plants. . . .	<i>Vicia Faba.</i>	<i>Phaseolus multiflorus.</i>	<i>Mathiola incana.</i>	<i>Caucalis anthriscus.</i>	<i>Saxifraga umbrosa.</i>
Hairs . . . .	—	—	Branched hairs; no colour except on the extreme outside; contents deep yellow.	No colour.	—
Epidermis . .	No colour.	No colour; no colour in cuticle.	No colour; cuticle brown.	Brown.	No colour.
Cork . . . .	—	—	—	—	—
Cortex . . . .	No colour.	No colour; contents dark brown and in some cases black.	No colour.	No colour; contents brown; outermost layer brown.	No colour; contents deeply coloured
Pith . . . .	No colour.	No colour.	No colour.	No colour; contents deep brown.	Deep reddish brown; contents also brown.
Sclerenchyma: a. Cortical. . . b. Stelar . . .	— —	— —	— Pale yellow.	No colour.	— —
Endodermis . .	Walls uncoloured; contents deeply coloured.	No colour; contents almost black.	—	No colour; blue contents.	—
Medullary rays.	—	—	—	—	—
Xylem: a. Primary . . b. Secondary .	Yellow.	Deep yellow.	Large vessels dark brown; small elements between bundles and between vessels yellowish brown.	Light brown.	Deep reddish brown.
Phloem . . . .	No colour.	No colour.	No colour.	No colour.	No colour.
Remarks . . .	All cell-contents were deeply coloured.	The examination was difficult owing to the large amount of iodine set free by the cell-contents.	The xylem became yellow in the sodium hydrogen phosphate solution.	Section very 'tender'; Epithelial cells of resin-ducts had deep brown contents.	The whole section was very yellow after the chlorine. In sodium hydrogen phosphate xylem and contents of many cells go deep reddish brown.

TABLE II (continued).

## C. AERIAL STEMS (continued).

	<i>Chaetanthus Chiri.</i>	<i>Helleborus viridis.</i>	<i>Lanum purpureum.</i>	<i>Euphorbia amygdaloides.</i>	<i>Buxus sempervirens.</i>
Plants . . . .	—	—	Slightly pink.	—	Outer layer of wall blue, rest uncoloured ; contents blue.
Hairs . . . .	—	—	No colour ; cuticle slightly pink.	No colour.	No colour.
Epidermis . .	No colour ; cuticle brown.	Brown ; cuticle deep brown.	—	—	—
Cork . . . .	—	—	—	—	—
Cortex . . . .	No colour ; contents deep brown.	No colour ; contents bluish brown ; some collenchyma present.	No colour ; contents brown.	No colour ; contents brown.	No colour ; contents yellow.
Pith . . . .	No colour ; contents deep brown.	No colour ; contents brown.	No colour ; the slight contents are brown.	No colour ; contents brown.	No colour ; contents yellow.
Sclerenchyma :					
<i>a.</i> Cortical . .	Brown.	—	—	—	—
<i>b.</i> Stellar . . .	—	—	—	—	—
Endodermis . .	—	—	No colour ; contents purplish red.	—	—
Medullary rays .	—	—	—	—	No colour ; contents blue.
Xylem :					
<i>a.</i> Primary . .	Brown.	Reddish brown.	Deep brown ; large vessels very dark brown.	Deep brown.	Yellowish brown.
<i>b.</i> Secondary .	Brown.	Not so red as the primary wood.	—	Deep brown.	Yellowish brown.
Phloem . . . .	No colour.	No colour ; contents bluish brown ; a specially wide band.	No colour ; contents yellow	No colour ; contents brown.	No colour.
Remarks . . .	There was much less colour in the young stems than in the older ones.	—	Small-celled tissue between bundles has uncoloured walls and yellow contents.	There are patches of cells above the bundles whose contents are very dark brown, probably lactiferous vessels.	A great deal of iodine set free by the cell-contents.

Plants . . . .	<i>Ligustrum vulgare.</i>	<i>Ulmus campestris.</i>	<i>Prunus amygdalus</i> (young).	<i>Prunus amygdalus</i> (old).	<i>Prunus amygdalus.</i>
Hairs . . . .	—	—	—	—	—
Epidermis . .	—	—	—	—	—
Cork . . . .	Outer layer reddish brown; other layers yellowish brown.	Deep yellow.	Deep yellow.	Deep yellow.	Brown.
Cortex . . . .	No colour.	No colour; contents deep brown.	No colour; contents deep blue.	No colour.	No colour; contents brown.
Pith . . . .	Very slightly yellow.	Slightly yellow.	Reddish brown; contents almost black.	Slightly yellow; walls sometimes pitted.	Thin-walled and pinkish brown.
Sclerenchyma : a. Cortical . . b. Stellar . .	— —	— —	— —	—	— Yellow; dark brown contents.
Endodermis . .	—	—	—	—	—
Medullary rays .	—	No colour; deep blue contents.	No colour; contents blue.	No colour; contents deep blue.	No colour; contents deep purplish blue.
Xylem : a. Primary . . b. Secondary .	Deep brown. Deep brown.	Reddish brown. Reddish brown.	Purplish pink. Purplish pink.	Pinkish brown. Pinkish brown.	Yellowish brown. Dull pink.
Phloem . . . .	No colour.	No colour.	No colour; contents blue.	No colour; rather a large amount present.	No colour; contents brown.
Remarks . . .	The contents of many cells were brown.	Layer of cells round the protoxylem had deep blue contents but no colour in walls. Xylem was dark brown in sodium hydrogen phosphate.	Xylem became pale pink in sodium hydrogen phosphate solution.	The ring of pith cells nearest the xylem has very deep blue contents.	Contents of pith cells near the xylem were purplish pink, as also were the contents of odd cells through the pith. Xylem became pinkish brown in the sodium hydrogen phosphate solution.

TABLE II (continued).

## C. AERIAL STEMS (continued).

Plants . . .	<i>Fagus sylvatica</i> .	<i>Populus balsamifera</i> .	<i>Salix alba</i> (young).	<i>Salix alba</i> (old).	<i>Prunus communis</i> .
Hairs . . .	—	—	Dark outer layer and inner layer; colourless middle layer.	Dark outer layer and inner layer; colourless middle layer.	—
Epidermis . .	—	—	No colour.	—	Brown.
Cork . . .	Brown.	Dark brown.	—	Deep brown.	—
Cortex . . .	Brown.	No colour; contents brown.	Slightly yellow; contents deep brown.	No colour; blue contents in layer of cells next cork; other contents brown or blue.	No colour; contents yellow.
Pith . . .	Yellow; contents almost black.	No colour; contents brownish blue.	Slightly brown.	No colour; blue or reddish brown contents.	No colour; very little, if any contents.
Sclerenchyma:					
a. Cortical. . .	—	—	Dark brown.	Deep brown.	—
b. Stellar . . .	Yellowish brown.	Brown.	Yellowish brown.	—	Very pale brown.
Endodermis . .	—	—	Brown.	—	No colour; contents brown.
Medullary rays .	No colour; contents black.	No colour; contents blue.	No colour; blue contents.	No colour; contents deep blue.	No colour; contents blue.
Xylem:					
a. Primary . .	Pinkish brown.	Brown.	Yellowish brown.	Brown.	Deep reddish brown.
b. Secondary .	Pinkish brown.	Brown.	Yellowish brown.	Three rings, brown, outer and inner darker than the middle one.	Reddish brown.
Phloem . . .	No colour.	No colour; contents often blue.	No colour; contents often blue.	No colour.	No colour; contents yellow.
Remarks . . .	Xylem became pink in sodium hydrogen phosphate solution. Isolated cells scattered through the xylem had blue contents.	—	Layer of pith cells adjacent to xylem had very deep blue contents.	Cambium was uncoloured. Layer of pith cells near xylem had very deep blue contents. A secondary root (cut longitudinally) was surrounded by these cells, but the main bulk of it was quite uncoloured.	Between xylem and pith were several layers of cells whose contents were dark blue.



Plant . . . .	<i>Corylus Avellana.</i>	<i>Stimera japonica.</i>	<i>Bauera rubioides.</i>	<i>Ribes nigrum.</i>	<i>Contradendia floribunda.</i>
Hairs . . . .	—	—	—	—	—
Epidermis . . . .	—	Pale yellow ; cuticle pale yellow.	—	—	Brown.
Cork . . . .	Brown ; outer and inner layers darker than the rest.	—	Brown.	Yellowish brown ; greenish towards the cuticle.	—
Cortex . . . .	No colour ; contents brown.	No colour ; blue contents in many cells.	No colour.	No colour ; brown contents.	No colour ; patches of small cells in angles of stem also uncoloured.
Pith . . . .	No colour ; blue contents, small in quantity.	No colour ; blue contents in many cells.	Brownish pink.	No colour ; contents yellow.	No colour ; granules in cells uncoloured.
Sclerenchyma : a. Cortical . . . . b. Stellar . . . .	Reddish. —	— —	— —	— —	— —
Endodermis . . . .	—	No colour.	Pink.	—	—
Medullary rays . . . .	No colour ; deep blue contents.	No colour ; deep blue contents ; specially large cells.	No colour ; purple contents.	No colour ; deep blue contents.	—
Xylem : a. Primary . . . . b. Secondary . . . .	Yellow. Spring wood pink ; autumn wood is yellow.	Brownish pink with some pink patches of cells. Autumn wood pink ; spring wood brownish pink.	Brownish pink. Bright pink (all three rings).	Dark brown. Bright pink (all).	Yellowish brown. Dark reddish brown, almost red.
Phloem . . . .	No colour ; contents yellow.	No colour.	No colour.	No colour.	No colour.
Remarks . . . .	Yellow after the chlorine ; pink in sodium hydrogen phosphate solution. Layer of pith cells near xylem had deep blue contents. Epithelial layers of resin passages were deep brown.	The xylem became brown in the sodium hydrogen phosphate solution.	The sections were green after the chlorine, and became bright pink (except the pith) in sodium hydrogen phosphate solution.	Sections became brown in sodium hydrogen phosphate solution. In very young stems the xylem was yellow and had no trace of pink, and the cambium had brown contents.	The section was rather 'tender'. Scattered through cortex and pith were bunches of crystals which did not become coloured. There were patches of small cells in the pith which were also uncoloured.

TABLE II (continued).

## C. AERIAL STEMS (continued).

	<i>Chrysanthemum indicum.</i>	<i>Caltha palustris.</i>	<i>Lamium album.</i>	<i>Rumex conglomeratus.</i>	<i>Brassica oleracea.</i>
Plant . . . .	—	—	No colour in either type.	—	—
Hairs . . . .	—	—	No colour; cuticle slightly pink.	No colour; cuticle brown.	No colour.
Epidermis . .	No colour.	No colour.	—	—	—
Cork . . . .	—	—	No colour; contents brown.	No colour.	No colour.
Cortex . . . .	No colour.	No colour.	No colour; contents brown.	No colour; contents brown.	No colour.
Pith . . . .	No colour.	No colour.	No colour; contents brown.	No colour; contents brown.	No colour.
Sclerenchyma:					
<i>a.</i> Cortical . .	Yellow.	—	—	Slightly yellow.	Pale pink.
<i>b.</i> Stellar . . .	—	—	—	No colour; blue contents.	Very strongly marked; no colour; bluish purple contents.
Endodermis . .	—	No colour.	No colour.	—	—
Medullary rays .	—	—	—	—	—
Xylem:					
<i>a.</i> Primary . .	Yellow.	Dark brown, almost black.	Dark brown.	Dark brown.	Dark brown.
<i>b.</i> Secondary . .	—	—	—	—	—
Phloem . . . .	No colour.	No colour; contents light brown.	No colour; contents yellow.	No colour.	No colour; yellow contents.
Remarks . . .	—	The sections were very tender.	—	The sections were very yellow after chlorination.	The sclerenchyma lay between and around the bundles, hence the difference in colour was strongly marked.

Plants . . . .	<i>Cytisus scoparius.</i>	<i>Syringa vulgaris.</i>	<i>Acer Pseudo-platanus.</i>	<i>Carpinus Betulus.</i>
Hairs . . . .	No colour.	Slightly pink.	—	Outer and inner layer of wall brown, middle layer uncoloured.
Epidermis . .	Bright yellow, including cuticle.	No colour ; cuticle brown.	Brown, including cuticle.	Brown.
Cork . . . .	—	—	Yellowish brown.	Yellowish brown.
Cortex . . . .	No colour ; contents very deep brown.	No colour, thick walls ; contents yellow or blue.	No colour ; contents brown.	No colour ; contents brown.
Pith . . . .	No colour.	No colour.	No colour.	No colour ; contents brown.
Sclerenchyma :				
<i>a.</i> Cortical . .	No colour.	—	Faintly pink.	—
<i>b.</i> Stellar . . .	No colour.	Pale pink.	No colour.	—
Endodermis . .	—	No colour ; blue contents.	—	—
Medullary rays .	No colour ; contents deep blue.	—	No colour ; contents deep blue.	No colour ; contents deep blue.
Xylem :				
<i>a.</i> Primary . .	Reddish brown.	Very red brown.	Deep pink.	Dark brown.
<i>b.</i> Secondary .	Reddish brown.	Very red brown.	Deep pink ; cells at any ring very strongly pink.	Very dark brown, almost black ; outer layer a redder brown than the rest.
Phloem . . . .	No colour.	No colour.	No colour.	No colour ; contents often nearly black.
Remarks . . .	A large amount of iodine was set free.	Outer layers of the cortex were so loose that they frequently stripped off. All colour effects were more marked in the older stems.	Xylem became yellow and then reddish in sodium hydrogen phosphate. In older stems, pith cells near the xylem were full of blue contents.	

TABLE II (continued).

## D. UNDERGROUND STEMS.

	<i>Bellis perennis.</i>	<i>Viola canina.</i>	<i>Fragaria vesca.</i>	<i>Anemone nemorosa.</i>
Plants . . .				
Hairs . . . .	—	—	Yellow.	—
Epidermis . .	No colour; cuticle deep yellow.	—	No colour.	Dark brown.
Cork . . . .	—	Dark brown.	—	—
Cortex . . .	No colour.	No colour; dark blue contents towards the outside.*	No colour; blue contents.	No colour.
Pith . . . .	No colour.	—	No colour; blue contents.	No colour; (collenchyma).
Sclerenchyma:				
<i>a.</i> Cortical . .	—	—	Yellow.	—
<i>b.</i> Stellar . .	—	Dark brown.	—	—
Endodermis . .	No colour; contents deep brown.	—	Yellow.	—
Medullary rays.	—	—	No colour; blue contents.	—
Xylem:				
<i>a.</i> Primary . .	Deep yellow. Deep yellow.	Only long radial areas of the xylem were coloured yellow; walls of cells in between were quite uncoloured, although they appeared to be lignified.	Deep yellow. Yellow.	Dark brown.
<i>b.</i> Secondary .				
Phloem . . .	No colour.	No colour; contents often blue; large amount of phloem.	No colour; blue contents.	No colour.
Remarks . . .	The sections were very 'tender'. The xylem became bright yellow in the sodium hydrogen phosphate solution.	* The cortical cells between the bundles radiated in a tangential direction from the patches of sclerenchyma almost as if they had been pulled out from these points. The sections were very 'tender'.	Sections were very yellow after the treatment with chlorine and became yellowish brown in the sodium hydrogen phosphate solution.	The contents of many cells in both cortex and pith were light brown, but the depth of colour was not so marked as was expected from the abundant cell-contents.

E. ROOTS.

	<i>Saxifraga umbrosa.</i>	<i>Vicia Faba.</i>	<i>Phaseolus multiflorus.</i>	<i>Euphorbia amygdaloides.</i>
Plants . . .	—	Outer layer of wall slightly yellow.	Outer layer of wall was slightly yellow.	—
Hairs . . .	—	Brown.	No colour ; cuticle brown.	No colour ; no colour in cuticle.
Piliferous layer .	No colour.	No colour.	No colour.	No colour.
Cork . . .	—	No colour.	No colour ; contents blue.	—
Cortex . . .	No colour.	No colour.	—	—
Pith . . .	Reddish brown.	No colour.	—	—
Sclerenchyma :				
<i>a.</i> Cortical . .	—	—	—	—
<i>b.</i> Stelar . .	—	—	—	—
Endodermis . .	—	—	—	Deep brown.
Medullary rays .	—	—	—	No colour ; contents blue.
Xylem :				
<i>a.</i> Primary . .	Reddish brown.	Dark brown.	Brown.	Very deep brown.
<i>b.</i> Secondary .	—	—	—	Brown.
Phloem . . .	No colour.	Reddish brown.	No colour.	No colour.
Remarks . . .	The sections became very yellow with the chlorine and turned reddish brown in sodium hydrogen phosphate solution.	Very few cells had contents, but where present these were coloured?	Pith appeared black to the naked eye in the sodium hydrogen phosphate.	

TABLE II (continued).

E. ROOTS (continued).			
Plants . . . .	<i>Helleborus viridis.</i>	<i>Ranunculus Ficaria</i> (tuberous root).	<i>Viola canina.</i>
Hairs . . . .	—	Outer and inner layer of wall brown, rest of wall not coloured.	—
Piliferous layer .	Dark brown; cells were irregular in shape.	Brown; particularly the radial walls.	—
Cork . . . .	—	—	Yellow.
Cortex . . . .	No colour; contents yellowish brown or blue.	No colour, but three rows of cells below piliferous layer had dark brown walls.	No colour; * contents blue.
Pith . . . .	—	No colour.	—
Sclerenchyma:			
<i>a.</i> Cortical . .	—	—	—
<i>b.</i> Stellar . .	—	—	—
Endodermis . .	Brown; especially deep in the radial walls.	Brown; radial walls especially dark.	—
Medullary rays .	—	—	—
Xylem:			
<i>a.</i> Primary . .	Deep reddish brown.	Dark brown.	Yellow.
<i>b.</i> Secondary .	Deep reddish brown, darker at each of the three rings.	—	Yellow, particularly strands of cells radiating from the centre.
Phloem . . . .	No colour.	No colour.	No colour; contents blue.
Remarks . . . .	—	—	* Certain patches of cells in the cortex just outside the vascular tissue were exceptional in that they had uncoloured contents.

The main object of this work was to determine whether the cellulose cell-wall contained sufficient protein to interfere with cellulose and pectin reactions, but the chloramine test produced such marked differentiation of colour in other tissues that it was decided to record the effects observed on all the tissues examined.

Parenchymatous tissue, whether of the cortex, cambium, or pith, never gave a positive reaction for protein ; the cell-walls remained quite colourless, although the lining layers of protoplasm, food granules, and starch all gave distinct colour reactions.

The cell-walls of the phloem were never coloured, although dark-brown or blue contents were often observed.

The endodermis and medullary rays were generally very distinct owing to the presence of starch which stained blue. The walls of the cells forming the medullary rays were quite colourless. In some cases the walls of the endodermal cells were brown, but in others no colour was developed.

The cell-walls of the pith cells, if thickened, generally set free some iodine.

Collenchyma has not been observed to give a colour reaction for protein in the plants examined up to the present.

The cuticle of the epidermis and suberized cell-walls, which are often brown in the natural state, deepened in colour with the chlorine and generally became darker still with the iodide.

Cell-walls containing lignin become bright yellow or bright brown with chlorine, deepening in colour in the sodium hydrogen phosphate solution, and becoming dark brown in the iodide.

In some cases a bright pink colour was produced instead of the customary brown. That is to say, plants differed one from another in the colour produced in lignified tissues, but for any one specimen the results were always the same.

With the xylem the following colour reactions were observed :

- (1) A uniform yellow or brown.
- (2) Bright pink.
- (3) The protoxylem dark brown and the rest pink.
- (4) The protoxylem dark brown and the rest light brown.
- (5) The spring and autumn wood may give different shades of colour.

Sclerenchyma may react in three ways :

- (1) It may be coloured like the xylem.
- (2) It may be coloured pink where the xylem is brown, and vice versa.
- (3) It may not be coloured at all.

It seems evident that these differences in the sclerenchyma and the xylem point to variations in the composition of the lignified cell-wall, as the experimental conditions were always the same.

The quantity of protein that could be detected in cellulose cell-walls by this method was investigated next, and the conditions that might exist in the cell-wall reconstructed in the following way :

Pure filter-paper (Whatman's No. 30) was immersed rapidly in aqueous solutions of gelatine of varying known strengths, laid on a glass surface, and a 'squeegee' roller was passed over the paper with some pressure. It was next submitted to the action of chlorine for  $1\frac{1}{2}$  hours, treated with potassium iodide solution, and the limits within which a brown colour could be detected observed both with the naked eye and with the microscope; in the case of the higher dilutions starch solution was employed to detect the iodine set free.

Under these conditions filter-paper absorbs its own weight of gelatine solution, so that by soaking it in these solutions a solution of gelatine in paper of the corresponding strength was obtained.

The filter-paper corresponds to the cellulose cell-wall, and the gelatine to the protein present in it.

The dilutions used and the results obtained are given in Table III.

In this table, except where otherwise stated, the results refer to the cell-wall of the tissue concerned.

TABLE III.

<i>Percentage of Gelatine in Paper.</i>	<i>Naked-eye Observations.</i>	<i>Observations with the Microscope.</i>
1.0	Deep red-brown colour.	Deep red-brown colour.
0.1	Lighter red-brown colour.	Lighter red-brown colour.
0.01	Pale yellowish brown colour.	Pale yellowish brown colour.
0.001	Yellow colour.	A pale yellow colour that could only just be detected.
0.0001	No colour, but just detected with starch.	No colour, but just detected with starch.
0.00001	No colour, either alone or with starch.	No colour, either alone or with starch.
0.000001	Ditto.	Ditto.
0.0000001	Ditto.	Ditto.

The experiments indicate that protein can be detected by this method at a concentration of 0.001 per cent., and possibly 0.0001 per cent. by the aid of starch.

In the preparation of a section the cell-wall is cut and consequently brought into closer contact with the reagents than in the case of the fairly complete cotton hairs in the paper, hence it seems likely that even a lower percentage of protein should be detected by this method.

In performing the experiments it is essential to remove all the chlorine from the paper, as otherwise this will set free some iodine. Exhaustion of the paper and rapid washing in distilled water immediately before treating with the iodide solution proved a satisfactory method.



That none of the colour produced was due to protein in the original paper was proved by chlorinating this, treating with potassium iodide and examining for iodine ; no trace of colour was produced.

This served also as a controlling test for the freedom of the paper from chlorine.

The experiments prove conclusively that a minute amount of protein in the cell-wall can be detected by this process, and this quantity is hardly likely to interfere with cellulose and pectin reactions.

The results of the investigation show that more than 0.001 per cent. of protein does not occur in the cellulose cell-walls of any of the plants examined ; and indicates that the amount is probably lower still, if indeed any is present.

#### SUMMARY.

1. A method has been developed for the detection of protein in the cell-wall, by the liberation of iodine from potassium iodide after treatment of sections with chlorine gas and sodium hydrogen phosphate solution. Where only traces of iodine were set free soluble starch was used for its detection.

2. For convenience and rapidity of chlorination a special apparatus was devised (Fig. 2).

3. Leaves, petioles, aerial and underground stems, and roots of various plants were examined by this method, with the results recorded in Table II.

4. Tests for oxycellulose both before and after chlorination were made ; the former provided further information of the occurrence of oxycellulose in the cell-wall, while the latter showed whether effective chlorination had taken place (Table I).

5. Lignified tissues give various results by this method, the colour produced being either yellow, brown or pink.

6. Quantitative experiments indicate that not more than 0.001 per cent. of protein occurs in the cellulose cell-wall of any of the plants examined, and in all probability the amount is lower still.

7. Cellulose cell-walls do not by this method show the presence of any protein. The amount of protein, if any, present in the cell-wall is unlikely to interfere with cellulose and pectin reactions.

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F.R.S., for suggesting the application of the chloramine test to this problem and the use of gelatinized filter-paper for determining the amount of protein; and to Professor Dame Helen Gwynne Vaughan for valuable help and advice during the progress of the work.

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# The Contractile Roots of *Oxalis incarnata*.

BY

D. THODAY.

With Plate XVII.

THE mechanism of the contraction of roots was first made the subject of careful investigation by de Vries (1). He selected for special study certain plants, belonging to different families of Dicotyledons (*Cynara scolymus*, *Verbascum thapsus*, *Conium maculatum*, *Dipsacus fullonum*), the thickened tap-roots of which show the phenomenon in a marked degree. The cambium forms in these roots an abundance of parenchyma. This parenchyma, especially the youngest zones near the cambium, was found by de Vries to be in a state of longitudinal tension, and he concluded that the seat of contraction was located there. He showed that this tissue and the individual cells composing it expand transversely, but shorten longitudinally with increase of turgor (and vice versa), and that the shortening as well as the transverse expansion which occur when the tissue is immersed in water may in part be permanent. The cumulative fixation of such changes, repeated in successive layers of secondary parenchyma, would account for progressive contraction during the growth in thickness of the root.

De Vries explained how diminution of length could accompany increase of volume of the cells on the hypothesis that the cell-walls are anisotropic, being more extensible transversely than longitudinally.

This explanation of the contractile mechanism has held the field ever since it was put forward by de Vries in 1880. It seems to fit most of the available facts.

It should be observed, however, that the examples specially investigated by de Vries are all similar in habit, and the roots are perennial organs with continued secondary growth. Among a wider range of plants for which he merely measured the contraction of roots immersed for a few days in water,

only one Monocotyledon is included (*Hyacinthus orientalis*). The highly specialized contractile roots, such as characterize some Monocotyledons and certain species of *Oxalis*, did not apparently come under his observation.

Subsequent investigators approached the subject almost entirely from a biological point of view. Daniel (2) describes the large fusiform roots of *Gladiolus* and *Crocus* without referring explicitly to their contraction in length, though he mentions the wrinkling which marks the commencement of 'resorption'. He regarded them as temporary storage organs which shrivel as their reserves of glucose are withdrawn. He states that glucose is abundant in the hypertrophied cortex and in the pith, but that when all the cells are flaccid and depleted none remains. These observations suggest that contraction may be correlated, in these plants, not with growth but with shrivelling of the parenchyma.

Rimbach (3-8), inquiring into the significance of the waving of the endodermal cell-walls (particularly of the Casparian strip) observed in the roots of certain Monocotyledons, traced it to contraction in length of these roots, and measured the extent and distribution of the contraction in a number of cases. A study of their anatomy and of the structural changes accompanying contraction satisfied him that de Vries's explanation was applicable, and that the cortical parenchyma is the active tissue.

In a certain class of cases, to which belong *Lilium martagon* and *Arum maculatum*, Rimbach draws attention to the fact that the diameter of the contractile root as a whole does not increase. The inner cells of the cortex grow at the expense of the outer, which collapse progressively, so making room for the transverse expansion of those within, which is a necessary condition of their longitudinal contraction (3, 8). In these roots the central cylinder remains straight, an observation which is in accordance with the position of the contractile tissue close around it.

So far, de Vries's explanation appears to fit Rimbach's observations.<sup>1</sup> In the case of the large contractile roots of *Oxalis elegans*, however, Rimbach records observations made by him in Ecuador, which appear in part to be at variance with contraction through the agency of growing cells. His interest had apparently been transferred ere this to the biological aspects of the subject, for he did not pay special attention to the mechanism of contraction in this case. He states that contraction, 'welche mehrere Monate in Anspruch nimmt, fällt zum Theile erst in die Zeit, in welcher Blätter und Fruchstengel schon im Welken begriffen sind und die neu angelegte Zwiebel ihre vollständige Grösse erreicht hat. Die Contraction jener Wurzeln dauert gewöhnlich noch an, wenn oberirdische Organe bereits nicht mehr vorhanden sind' (5, p. 93). Since the aerial parts die down at

<sup>1</sup> Arber's recent observations (11, pp. 19-21) indicate that further work on the Monocotyledons generally is nevertheless desirable. M. B. Church (10) has also expressed difficulty in following de Vries's hypothesis.

the onset of the dry season it seems that contraction must in part coincide with the drying of the soil. Elsewhere he states that the part of the root that is growing in thickness shortens at the same time ('gleichzeitig') very strongly, so that it becomes straight and taut, the maximum contraction observed for a length of 5 mm. being 70 per cent.; but he adds: 'Anfangs fallen Dickenzunahme und Verkürzung der Wurzel zusammen, *zuletzt aber wird die Wurzel wegen des Schrumpfens der Rinde wieder dünner während die Verkürzung in ihr noch fort dauert*' (6, p. 151) (italics mine). It is not clear on what evidence Rimbach bases his statement that extensive contraction accompanies thickening of the root. Whether it is true or not, however, we have recorded in these two quotations a correlation between the later stages of contraction and, on the one hand, the wilting and withering of the aerial parts of the plant and, on the other hand, the shrivelling of the parenchymatous tissues of the root itself.

Rimbach also observed that the 'central cylinder' becomes much bent and distorted ('schr verbogen') in the process. It may be remarked at once that distortion and zigzag bending of the core of xylem is hardly compatible with the localization of an actively shortening tissue close around it, which would tend to keep it straight (as in the case of the Monocotyledons already mentioned); while, since the outer parenchyma shrivels in the later stages of contraction, we cannot look there either for an actively growing tissue of the character postulated by de Vries.

Another species, *Oxalis cernua*, a native of the Cape, which also forms fusiform contractile roots, has been studied by Ducellier (9) in Algeria, where it is a common weed in gardens and orange groves. From his detailed account of the life-history of this species it appears that the contractile root is formed early, at the base of the old bulbil from which the plant arose, and begins to swell soon after the leaves appear above the ground. The mature '*tubercule*' has a sugary sap and a water-content of up to 90 per cent. Its contraction coincides with the growth of a new bulbil near its summit, just below the old bulbil. Ducellier implies that it serves a double function—it is an ephemeral storage organ (lasting two to three months) which empties, wrinkles, and soon disappears during the formation of the new bulbil, at the same time contracting in length and drawing the bulbil down into the soil (cf. also Marloth (12), pp. 318–19). Here more plainly still we have to deal with a contraction in length which accompanies shrivelling, not growth.

#### *Oxalis incarnata.*

Another Cape species of *Oxalis*, *O. incarnata*, has been the subject of a general investigation by Miss M. Copland, B.Sc., in this laboratory, and I am indebted to her for information as to its life-history here which has facilitated this special investigation. The species is established in a garden

bed near by, reproducing fairly freely by bulbils, and it forms contractile roots several centimetres long and 5 mm. or more in diameter. Examination of a longitudinal section through a contracting root showed that the contraction is a very different process from growth contraction as described by de Vries. Where the external surface of the root was strongly wrinkled the most conspicuous feature was an alternation of transverse plates of turgid cells with layers of collapsed cells, which appeared as if crushed between the still turgid layers.

When the contraction is well advanced it is easily seen that zones of collapse extend more or less continuously through the parenchyma right across the root. For growth contraction of the type described by de Vries longitudinal continuity of the active tissue is essential. No such continuous turgid parenchyma exists in the contractile root of *O. incarnata* at the stage referred to, although contraction is still in progress.

The parenchyma of the swollen root, which is referred to by Rimbach (*O. elegans*) and Ducellier (*O. cernua*) as cortex, is in *O. incarnata* formed by the vascular cambium, and corresponds to secondary phloem. Slender strands of narrow elements, including sieve tubes, traverse it longitudinally. As described by Ducellier for *O. cernua* it is translucent, and the core of xylem is visible when the root is held up to the light. In sections of the living root no air-spaces can be detected. The parenchyma has the appearance of a water-storing tissue. Reducing sugar is present in some quantity (cf. *O. cernua*, p. 573, above). The sap from a piece of root, boiled and crushed in a small volume of water, gave a copious precipitate with Fehling's solution. A rough quantitative estimation with another root weighing 0.95 gr. gave about 2.8 per cent. reducing sugar estimated as glucose. Preliminary qualitative tests indicated the presence of both glucose and fructose, but not cane sugar.

In sections from a region just beginning to contract, chlor-zinc iodine differentiates very clearly the layers of turgid cells, with protoplasm stained yellow, from the intervening purplish zones of cells beginning to collapse. In the latter the protoplasm has disappeared and no longer masks the cellulose reaction of the thin walls.

The cell-walls also undergo a change. In the living cells they stain more deeply with Delafield's haematoxylin, and the numerous pits show up very clearly, especially in walls from which the protoplasm has contracted. The walls of collapsing cells stain relatively slightly, and the pits are no longer visible. The chemical nature of the change is still obscure; no difference has so far been detected in their behaviour to pectic or cellulose stains.

As contraction proceeds the zones of collapsed cells include more and more layers. No clear evidence of contraction unaccompanied by collapse of cells has so far been found, such as would be afforded by downward dis-

placement of the old bulbil, the scales of which remain attached for a long time in this species, or by the sinuous course of vessels.

The core of xylem of the fully swollen root is not quite straight, but at the point of attachment of the xylem of each lateral root is bent outwards towards the latter, forming a kink (Pl. XVII, Figs. 2 and 3). At the point of exit the periderm is held inwards correspondingly. It may be inferred, therefore, that the xylem of the lateral root has hindered the transverse growth of the parenchyma of the main root. As laterals occur at irregular intervals, on different sides, a number of kinks in different directions are impressed upon the woody core, which predetermine the chief angles of its later very pronounced zigzag course when contraction is far advanced (see Pl. XVII, Figs. 1, 2, and 4). Actual longitudinal compression is then also evidenced by the approximation of reticulations or spirals, and more especially by the independent sinuosities of the older vessels.

Contraction thus involves a progressive depletion and collapse of the bulk of the parenchyma and a compression of the more resistant woody core.

#### *The contracting force.*

For an understanding of the mechanism of contraction, the following facts stand out as most significant :

- (1) The collapse of parenchymatous cells by withdrawal of their sap after the disappearance of their protoplasm.
- (2) The orderly progress of this depletion.
- (3) The persistence throughout of plates of turgid cells which are at first transverse, though, as will be explained later, they afterwards become oblique.

The withdrawal of sap gives the clue to the nature of the contracting force. The sap is being transferred in part to stem and leaves, while these remain, in part to the developing bulbil. The absorption of water by the bulbil and, during transpiration, by the aerial shoot will result in a water deficit, a reduced turgor pressure of the living cells, and diminished pressure or even cohesion tension in the vessels. The whole root, including the water tissue, will be affected as if subjected to increased atmospheric pressure, or as if a pull were exerted on the periderm at all points, tending to reduce the volume of the enclosed tissues. The latter way of looking at the situation becomes especially appropriate if and when an actual cohesion tension is transmitted through the xylem.

Against such contracting forces the root is supported in the transverse direction by the radially continuous plates of cells that maintain their turgescence. These cells will only yield water until their power of holding it has increased sufficiently to balance the opposing forces ; that is, until the tension in their walls has relaxed so far as to bring the residual turgor

pressure and the cohesion tension acting together on the one side into equilibrium with the increased osmotic pressure of their sap on the other.

The layers of cells that lose their protoplasm, on the other hand, can no longer hold their sap osmotically. The walls are permeable to solutes as well as to water, and the sap will be gradually withdrawn as such through the xylem towards the part where the water deficit is initiated. Any removal of solutes by way of the phloem will expedite the withdrawal of water by way of the xylem.

The only obstacles to the withdrawal of sap, and hence to the longitudinal contraction of the root as a whole, are the resistance to deformation offered by the walls of the collapsing cells themselves, by the periderm and by the xylem, together with the frictional resistance of the soil.

The resistance of the soil to downward movement of the periderm accounts for the following phenomenon: Before contraction begins, the cells of the parenchyma are seen in radial section to be arranged in transverse rows (Pl. XVII, Fig. 3). On the other hand, in the upper part of a root beginning to wrinkle, the rows are oblique, sloping down towards the core (Pl. XVII, Figs. 1 and 2). The frictional resistance of the soil must therefore be greater than the resistance offered by the xylem to compression. The change of slope ultimately reduces the diameter of the root very appreciably (cf. Pl. XVII, Figs. 1 and 2): the surface is thus withdrawn from close contact with the soil.

The water deficit, and with it the cohesion tension, must often fluctuate considerably over a period of weeks or months occupied by the contraction of the root. The turgescient cells will accordingly expand and contract. There are indications that they may even grow a little (compare the sizes of the cells in Pl. XVII, Figs. 1, 2, and 3). There should, in fact, be no serious obstacle to be overcome (if they are still capable of growth) when an improvement in the water-supply relaxes the cohesion tension, at any rate after the surface of the root has been withdrawn from close contact with the soil in the manner just described. The collapse of the intervening layers, on the contrary, will be mainly irreversible and so cumulative and progressive.

There must of necessity be accommodations in detail between the tissues during contraction. The cambium is plastic, for it retains its normal appearance, and at all stages of contraction vessels may be found which, by virtue of their straight course and uncompressed structure, appear to have been recently differentiated. How the cambium adapts itself to the change of length, whether by sliding growth or in some other way, requires further investigation.

In parts, particularly at an early stage, the strands of secondary xylem may appear to prevent localization of collapse in the innermost parenchyma adjoining them. In such cases a longitudinal section at first sight suggests



that the inner, younger phloem parenchyma might be an actively shortening tissue. Continuity in a straight course is, however, lacking. Careful observation shows a gradual transition from the collapsed layers farther out; here and there fine folding of walls can be recognized; while, at a later stage, it is very evident that the strands are mostly oblique to the direction of contraction, and that when this is not so the xylem begins to give way and the zones of collapse to extend right through the adjacent parenchyma.

In roots which have been well supplied with water for some time after contraction was first initiated, so that contraction is slowed down or inhibited, the cambium may continue its activity. In this case each strand of secondary xylem and associated cambial tissue appears to behave as a more or less independent unit in which the cambial arc adds new xylem within and forms new small-celled tissue without. As xylem is formed more actively where the strand is bent inwards, the cambial zone tends to straighten longitudinally. When contraction is proceeding rapidly without interruption this straightening process either cannot go on or cannot keep pace with the buckling effect of compression; but in some fully contracted roots, with the mature parenchyma completely shrivelled, the strands appear to have grown in thickness and straightened in the way just described. In such cases the straightening process may well have been assisted by the pressure of the collapsing parenchyma; for in the last stages the few remaining plates of turgid cells come to lie almost alongside the core, and no longer prevent the compressing forces acting radially and more or less uniformly.

#### *Experimental evidence.*

The experiments to be described were made with the object of testing the explanation of contraction just outlined.

#### *Experiment I.*

Two plants with well-swollen roots were planted in a glass-sided box, with the roots, marked at intervals with Indian ink, behind the glass and separated from the soil by a layer of blotting-paper. Wrinkling had not begun when the experiment was set up.

*Plant A* had a root swollen for about 3 cm. to just over 5 mm. in the widest part. After twelve days (Table I) the uppermost 8 mm. had contracted in length 37 per cent. and in diameter about 5 per cent. The next 8.6 mm. had contracted 18 per cent. in length, the remainder not at all, while the diameter of the uncontracted part had increased by up to 25 per cent.

After a further twenty-two days contraction had not proceeded much farther, while the lower part had swollen still more. There is indication of a recovery from the previous slight contraction in diameter of the uppermost part, but the measurements are rough. New rootlets had been produced,

both by the slender branching lower parts of the contractile root itself, and by the absorbing roots attached above it to the stem. It seems clear, therefore, that contraction occurred chiefly while the water-supply was inadequate, immediately after replanting. During this period most of the leaves were shed. When the absorbing system began to function again contraction in length slowed down, and the lower part was able to grow in thickness. The small contraction still registered in the upper part suggests the consideration that, once cells have lost their protoplasm, they will continue to yield water to balance a water deficit in other cells or tissues; growing cells in the lower part could therefore draw upon those zones in the upper part in which collapse has been initiated but is not yet complete; but it is doubtful whether this is ever under normal conditions a significant factor in the mechanism of contraction. The results for plant B show rather that when the lower part of the root has no absorbing roots to supply it with water, it is unable to grow at the expense of the upper part.

*Plant B* had a root thickened for 6 cm., and slightly stouter than that of plant A. In twelve days the uppermost 18 mm. contracted 47 per cent. in length and about 10 per cent. in average diameter; the next 18 mm. contracted 25 per cent., the next 10 per cent. in length, without significant change in diameter. During the next twenty-two days contraction proceeded farther, especially towards the tip. The absorbing roots of this plant had all died and had not been replaced. The tapering tip of the swollen root had begun to shrivel up.

The contrast between the behaviour of the roots of these two plants is clearly in accord with the view that contraction depends upon a water deficit.

### *Experiment II.*

A plant with a contractile root about  $2\frac{1}{2}$  cm. long, of which the uppermost 2 mm. were already wrinkled, was set up with the root (marked with Indian ink) enclosed in moist air in a corked tube with a little water at the bottom. During the first two days the plant was exposed to the sun, but was transferred to the shade on the morning of the third day, as the leaves had wilted. Measurements showed contraction to the extent of about 50 per cent. in the upper half, and a shrinkage in diameter of 20 to 15 per cent. The periderm was wrinkled, though not as closely as in roots contracted (less rapidly) under natural conditions. Longitudinal sections showed the usual appearance of zones of collapsed cells alternating with still turgescient cells; but the rows were not oblique, contraction having been free from the external constraint of friction with soil.

In this case, contraction, showing features essentially similar to those associated with contraction under natural conditions, has resulted from withdrawal of water by transpiration unbalanced by absorption. One significant

TABLE I.

1925	Lengths of Successive Zones of Root from Base downwards, in Millimetres.				Diameter at Lower End of Zone, in Millimetres.			
	Aug. 6.	Aug. 18.	Sept. 9.		Aug. 6.	Aug. 18.	Sept. 9.	
Root A.	4.0	2.3	2.0	Strong contraction.	5.3	5.0	—	Growth nil.
(Absorbing root system survived transplanting and grew.)	4.0	2.7	2.3		5.2	4.9	5.2	
	4.1	3.3	2.8		4.8	4.7	5.2	
	4.5	3.7	3.4	Contraction nil.	3.6	4.3	5.0	Growth in diameter.
	5.0	5.0	4.6		3.2	4.0	5.0	
	5.7	5.7	5.6		2.1	2.1	3.0	
Root B.	5.5	4.0	4.0	Strong contraction in length.	5.9	5.0	—	Contraction in diameter.
(Absorbing root system ceased to function.)	5.3	1.6	1.3		5.8	5.0	—	
	4.4	2.4	1.7		5.5	4.8	—	
	2.5	1.3	1.1		4.2	4.0	—	
	3.4	2.6	1.9		3.6	3.4	—	
	5.9	3.9	2.2		3.9	3.7	3.8	
	4.0	3.1	2.5		3.3	3.0	3.7	
	4.8	4.0	3.5		3.0	3.0	3.2	
	4.0	3.8	3.8		2.8	2.8	3.1	
	5.2	4.5	4.0		2.9	2.8	3.1	
	4.0	3.4	2.0		2.9	3.0	3.1	
	4.2	4.0	3.3		2.7	2.6	2.3	
	5.3	4.6	2.7		1.3	1.8	(Shrivelled.)	

point of difference may be recorded. The wrinkling and associated contraction were exhibited more or less uniformly by a full centimetre of the root, and marked contraction occurred over the whole measured length of more than 2 cm. within three days. Under normal conditions the process is slower and better controlled, so that a clear gradation is observable, from the strongly contracted upper region through less and less wrinkled to quite smooth zones.

### Experiment III.

Nine roots of six plants were marked and treated in various ways, as detailed in Table II: some with, some without bulbils or aerial shoots; some wholly submerged, others with only the fibrous lower parts submerged; some robbed of their absorbing system. The detailed measurements are not given, but the measured zones have been grouped into lengths approximating to 2 cm., and the results stated as percentages of the initial lengths.

The data have been arranged in order of magnitude of the figures for the length of the upper 2 cm. after thirteen days, i.e. in the order of increasing contraction of the upper part of the root. On the whole this order follows increasing demand and decreasing supply of water. The results thus provide further confirmation of the dependence of contraction on diminishing water-content.

A more detailed analysis of these results would hardly be justified, as the number of roots treated in each of the various ways is too small. A few points seem nevertheless to be worth comment.

TABLE II.

<i>Experiment III.</i>		<i>Length of Zones about 2 cm. long, per cent. Original Length.</i>				
<i>Begun 11th Sept. 1925.</i>		<i>After 13 days.</i>		<i>After 76 days.</i>		
<i>Plants in greenhouse in shade under staging, in tubes covered with tinfoil.</i>		<i>Top 2 cm.</i>	<i>Next 2 cm.</i>	<i>Top 2 cm.</i>	<i>Next 2 cm.</i>	
5. Submerged: bulbil only (above water).		99	99	—	—	Decaying.
2 a. Submerged: bulbil and shoot.		96	98	93	97	
2 b. Nearly submerged: attached above bulbil to same shoot as 2 a.		94	—	94	—	
1 a. Above water; absorbing system below in water: bulbil only.		92	98	74	94	Shoot had grown out from bulbil.
4. Above water; absorbing system below in water: bulbil and shoot: absorbing roots above bulbil in water in a second tube.		88	97	76	89	
6 a. In air, detached, absorbing system cut off, no water.		86	84	—	—	Shrivalled.
6 b. Ditto.		85	96	—	—	Shrivalled.
1 b. Above water; absorbing system below in water: attached to shoot (bulbil and principal root detached = 1 a).		83	—	67	—	
3. Submerged: bulbil and shoot system (above water): absorbing roots removed.		81	85	58	55	Three new slender roots 3-5 cm. long had grown out from cut end.

Roots 6 a and 6 b show contraction through loss of moisture from the surface of the detached roots enclosed in a dry tube plugged at both ends with cotton wool. A similar though smaller loss of water will have occurred in the case of other roots not submerged in water: the contrast between 2 a or 2 b and 1 b illustrates this. On the other hand root 3, after detachment of the absorbing roots, did not absorb through the periderm enough water to replace the loss by transpiration from a meagre, soon leafless shoot system.

The contraction of root 1 a, coinciding with the production of a new aerial shoot from the bulbil, is of interest, because it draws attention to the storage function of the swollen root. Sugars present in the collapsing cells are doubtless drawn upon. This is prepared for by the disappearance of protoplasm from the cells.

*The contractile mechanism as an adaptation.*

It is hardly necessary to emphasize that the contraction of the swollen root of *Oxalis incarnata*, which it is the object of this paper to elucidate, is not merely the direct mechanical effect of the absorption of water by the

transpiring shoot or by growing parts. The withdrawal of water (or sap) is preceded and regulated by the disappearance of the protoplasm from the cells, which later collapse in an orderly sequence. The forces which bring about the withdrawal of the water bring about at the same time the contraction of the root, but this contraction is conditioned by the behaviour of the parenchyma.

It appears from a perusal of the experiments already described that uncompensated withdrawal of water by the transpiring shoot is enough to start the process. In other cases it is connected with growth of a bulbil or a new shoot; but, as already pointed out, there must in all cases be withdrawal of reserves as well as water.

An analogous phenomenon is presented by detached shoots of many species of *Mesembryanthemum*. Apical growth continues and turgor is maintained at the expense of water and substance withdrawn from the oldest leaves in succession. The diminishing water-content of the shoot as a whole is not borne equally nor in proportion to their youth and vigour by all parts, but definite parts in definite order yield their water completely to make good the deficiency in the remaining parts.

Phenomena of this kind can only be described at present in terms of such conceptions as 'behaviour' and 'correlation'. The evolution of such modes of reaction to environment is very difficult to conceive on a purely mechanistic basis. There is nothing in the small difference of age between one *Mesembryanthemum* leaf and the next adequate to account for the sharp contrast in their behaviour. It is even clearer, in the case of the contracting root of *Oxalis incarnata*, that there is no difference of age and no apparent difference in structure to account for certain layers of cells yielding up their contents, while adjacent layers retain their contents and remain turgid.

A comparative study of other species of *Oxalis* may perhaps throw further light on this puzzling aspect of the mechanism. Yet, while we are so much in the dark as to the mechanism even of translocation, it is not to be wondered at that the more subtle regulatory mechanisms elude us. Meanwhile it must remain a matter of opinion whether the peculiar behaviour of the parenchyma took its origin in some chance redistribution or modification of genes, or was acquired as a habit on the lines of the mnemonic hypothesis.

One thing seems clear: just as the swelling of the root precedes its contraction, so must we suppose that the swollen root was first evolved as an evanescent storage organ and that afterwards it became further specialized, the function of contraction being superposed upon that of storage. It is to be remembered in this connexion that the storage of water is of no less importance than the storage of food to plants growing in a climate, like that of the Cape, marked by hot, dry seasons (cf. Marloth (12), pp. 314-19).

Large quantities of water are stored in the subterranean perennial parts, some of them of great size, of many Cape plants, to be drawn upon usually in the flowering season in spring or early summer.

The Cape species of *Oxalis* mostly flower as well as vegetate in the winter season, beginning as early as April or May, and die down at the approach of the dry season. They do not all form swollen roots, and it would be interesting to discover whether these roots when formed are always contractile, or are sometimes simply storage organs. *O. incarnata* is one of the latest to flower, usually between August and December, though occasionally earlier. No details are available for this species in its native habitat, but the water and food stored in the swollen root probably serve, as in the case of *O. cernua* (Marloth (12), pp. 318–19), chiefly for the formation of bulbils, or for the completion of this process, at the onset of the dry season. Against a sudden early drought it would act as a valuable safeguard.

#### SUMMARY.

The shortening of contractile roots is brought about, according to de Vries, by growth in parenchymatous cells with anisotropic walls which are more extensible transversely than longitudinally.

The mechanism of contraction appears to be of quite a different kind in the case of *Oxalis incarnata*. In the specialized swollen roots of this plant contraction in length is correlated not with growth but with a shrinkage in volume, owing to the withdrawal of sap from transverse zones of cells in the storage parenchyma, preceded by the disappearance of protoplasm from these cells.

Between the zones of collapsing cells, transverse plates of turgescient cells which have retained their protoplasm prevent radial contraction, except in so far as they later become oblique to the axis owing to friction between the periderm and the soil.

Experimental data illustrate the dependence of contraction on a water deficit.

The contracting force is therefore atmospheric pressure acting on the outside of the root, unbalanced by an equal pressure in the vessels, or atmospheric pressure together with a cohesion tension transmitted in the xylem from the transpiring aerial shoot or a growing bulbil.

These forces are brought into action through the disappearance of protoplasm from the cells in an orderly sequence. This peculiar behaviour may apparently be initiated in response to a diminution of the water-content, or be correlated with the growth of a bulbil or aerial shoot.

The essential difference between the contractile root of *Oxalis incarnata*

and a root of similar form serving merely for storage would seem to lie in the specialized behaviour of its parenchyma. No initial structural difference has been detected between those cells which first collapse and those which longest maintain their turgescence.

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#### EXPLANATION OF PLATE XVII.

Illustrating Professor D. Thoday's paper on the Contractile Roots of *Oxalis incarnata*.

Photographs of microtome sections through three regions of a contracting root already strongly wrinkled at the top.

Figs. 1 and 2. Two stages in contraction, showing alternating zones of turgescence and collapsing cells in the parenchyma and the irregular course of the xylem strands. *r.*, lateral root.

Fig. 3. Swelling nearly complete, contraction not yet begun. The xylem strands follow a much more regular course, with kinks at the points of exit of lateral roots (*r.*).

Fig. 4. A portion of a section, parallel to that shown in Fig. 2, more highly magnified.

(Magnifications : Figs. 1-3,  $\times 15$  ; Fig. 4,  $\times 27$ .)







1.



2.



3.



4.

Root coll



# Choreocolax Polysiphoniae, Reinsch.

BY

H. H. STURCH.

With fifteen Figures in the Text.

*CHOREOCOLAX POLYSIPHONIAE*, parasitic on *Polysiphonia fastigiata*, Grev., was originally described and figured by Reinsch (1) in 1875, and is mentioned as the first investigated of this group of minute cushion-shaped holoparasitic Florideae, of which *Harveyella* is perhaps the best known genus (2). In his plate Reinsch figures sections of what is apparently a young plant just before the production of antheridia. The next mention of *Choreocolax* is by Farlow (3), when the tetraspores are shown for the first time. In 1889 the species was mentioned by J. Reinke (4) in a note on *Harveyella mirabilis*, and in the same year Schmitz (5) placed *Choreocolax* next to *Binderella* in the Gelidiaceae. The genus was also mentioned by Batters in his list of Berwick Algae. The first description and figures of the cystocarp appeared in a paper by H M Richards (6), in which the structure of the plant and that of the mature cystocarp were partly described and figured, but the details of development from the carpogonium to the completed cystocarp were not observed. It was suggested that the plant should be removed from the Gelidiaceae, and placed in the Chaetangiaceae near *Galaxaura*. The antheridia were mentioned by Buffham in the same year.

In 1896 Schmitz (7) again placed *Choreocolax* in the Gelidiaceae near *Binderella*. His account gives small diagrammatic figures of the antheridia, a carpogonial branch, and two cystocarps. The question of the auxiliary cell is left undetermined, and as the description of the plant differs in many important respects from the much fuller one previously given by Richards, it was considered that the parasite was worth further investigation.

*Choreocolax* is also included by de Toni (8), and the above-mentioned papers, together with its mention by Borgesen, Cotton, Kylin, and other authors, show that the plant has been found at the Faroes, Clare Island, Shetland, Scotland, Bangor, and various parts of N. America, and generally on the coasts of the North Atlantic. I had failed to find the plant either

on the south coast of Ireland, near Southsea, or at Plymouth, until Professor R. W. Phillips, in May 1924, very kindly sent me some specimens of *Polysiphonia fastigiata* bearing small cushion-shaped objects, which on examination proved to be *Choreocolax Polysiphoniae*. In June of the same year I discovered them in abundance near Devil's Point, Plymouth.

The two other species of the genus, *Ch. tumidus*, parasitic on *Ceramium involutum*, mentioned in Batters's Catalogue of British Marine Algae, and *Ch. Cystoclonii*, parasitic on *Cystoclonium purpurascens*, I have not yet been able to find.

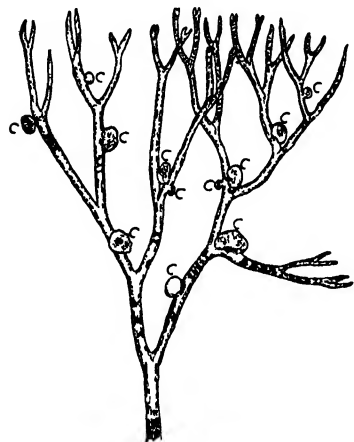


FIG 1. *Polysiphonia fastigiata* bearing *Choreocolax Polysiphoniae* (c).

As *Choreocolax* is abundant within ten minutes' walk of my quarters, it has been possible to examine fresh living specimens throughout the year. The plants have been cut at once by hand and the sections examined in sea-water, thus the distortions produced by various preserving and mounting methods have been eliminated. The most successful methods of preservation for sectioning later on were the use of the ordinary chrom-acetic fluid, or of alcohol and glycerine. After these, the distortion could be corrected to a considerable extent by lactic acid as a mountant. Sections of

preserved material were usually cut in frozen mucilage. No difficulty was found in applying the ordinary paraffin methods to the host plant, but the parasite was so distorted that the sections showed little resemblance to those of the living plant, and no useful information could be obtained from them.

*Choreocolax Polysiphoniae* bears its antheridia, procarps, and tetrasporangia invariably on separate plants, and no signs of abortive tetrasporangia have been found on other individuals. *Polysiphonia fastigiata* was collected in small and approximately equal quantities each fortnight throughout the year, from any rock in one small bay, and numerous specimens of *Choreocolax* were obtained each time (Fig. 1).

Antheridial, cystocarpic, and tetrasporangiate plants were found in approximately equal quantities at each collection. Perhaps the number of very young cystocarps was a little higher in April than at any other time, but evidently *Choreocolax* is not much affected by the variations of light or temperature in this district.

*Choreocolax* and *Harveyella* are much alike in size and external appearance. The external soma of *Choreocolax* averages from 300 to 400  $\mu$  in height, and its greatest width from 300 to 800  $\mu$ . Slight differences in

colour between the three parasites become evident in examining large numbers of living specimens; *Harveyella pachyderma* is always an opaque milk-white, *H. mirabilis* white but less opaque, occasionally brown, and *Choreocolax* is usually brown, but sometimes translucent and colourless. All three, if kept for a few days in unchanged sea-water, or for a few minutes in any other liquid, become brown; colouring matter is taken up by the gelatinous outer membrane of the parasite, probably from the dead cells of the host.

The situation of *Choreocolax* is of interest, for it is completely parasitic on *Polysiphonia fastigiata*, which is itself hemiparasitic on *Ascophyllum nodosum*. The two may be regarded as exemplifying, the latter the commencement, and the former the limit of parasitism in the Florideae.

*Choreocolax* and *Harveyella* were, in the very distant past, Florideae each with a fully elaborated reproductive cycle of three generations, gametophyte, carposporophyte, and tetrasporophyte, and were probably comparatively small plants with inferior somatic development. Like so many other of the smaller Florideae, unable to meet the competition of stronger plants in the struggle for suitable substratum on which to grow, they were forced to become epiphytes or perish. If the cortical protection of the host in any part of its surface happened to be easily penetrable, the epiphyte might sink ramifications to obtain stronger attachment. If these attachment organs could absorb food material, the intruder would commence its career as a parasite. The future depended on the suitability of the food material found and the relative metabolic efficiency of the two plants. The would-be parasite might find itself entirely unable to make use of the food found, or the host might be able to take food from the intruder; in either case the latter would remain an epiphyte or die. In the case of *Choreocolax*, a Floridean parasitic on a Floridean, the food material was suitable, and the parasitism has been so successful that it has been carried nearly to its extreme limit. Photosynthesis has been lost, and the free soma has dwindled until it occupies a space scarcely larger than that taken up by its own haustorial filaments inside the host (Fig. 2).

In fact the free soma is only large enough to contain the cystocarps. As a reproductive machine *Choreocolax* is extraordinarily efficient, a very large number of spores of both kinds being produced with as little somatic growth as possible.

Although during so much of its life above sea-water level, it never suffers from desiccation, the closely pressed bunches of its host on the hanging fronds of *Ascophyllum*, even at low tide on the hottest days of summer, being still surrounded by a film of water; while every mature *Choreocolax* has its whole surface covered with antheridia or tetrasporangia, or again contains as many cystocarps as the vegetative thallus can enclose.

In the case of *Polysiphonia fastigiata*, a Floridean parasitic on a Phaeo-

phycean *Ascophyllum nodosum*, the food material is unsuitable, and *Polysiphonia* has only been able to become partially parasitic. Hence it has retained its somatic equipment undiminished. It has attained a position of optimum exposure to sunlight and free oxygen, and is evidently very successful, since it is abundant throughout the year.

That *Polysiphonia fastigiata* is able to absorb some food material from *Ascophyllum* has been conclusively shown by L. Batten (9), not only by the

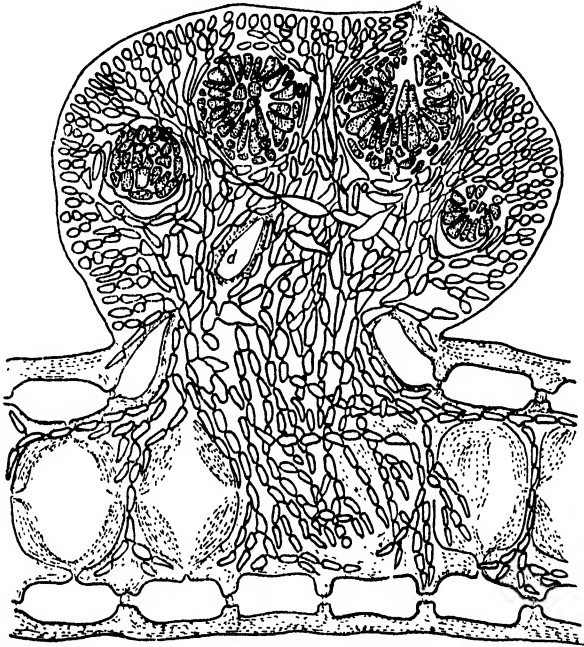


FIG. 2. Section of *Choreocolax* on *Polysiphonia*, with carposporophytes in various stages of development and cut at various angles. The cells of the carposporophytes are dotted. *d*, cell of host plant carried up by growth of parasite.

changes in the contents of the host cells which happen to be in contact with the haustoria of the *Polysiphonia*, but also by the inferior growth of the rare specimens of *P. fastigiata* which grow directly attached to rock.

When a section of *Ascophyllum* containing haustoria of *Polysiphonia* is treated with a saturated solution of vanillin in strong hydrochloric acid, and then immediately examined in the same solution, the cortical cells of the *Ascophyllum* show the characteristic crimson physodes of these plants, except in those cells which are in contact with the haustoria of the *Polysiphonia*. In these cells the physodes have almost entirely disappeared, a collapsed mass of cell-wall remaining (Fig. 3).

As little food material can be absorbed and used, *Polysiphonia* not only remains normal in size, but retains its photosynthetic layer unimpaired. It is a hemiparasite still remaining in the first stages of its career.

In this paper the term *procarp* is used to connote the cells of the carpogonial branch, including the carpogonium and its accessory trichogyne, but not the auxiliary cell or the mother-cell of the latter, even when these are combined into a group with the carpogonial branch; and by *auxiliary cell* is meant that somatic cell into which the zygote nucleus is transferred, and from which the gonimoblasts arise, as distinguished from any somatic cell with which the carposporophyte fuses and from which it abstracts food. These were also called 'auxiliary cells' by Schmitz, but to make the story quite clear I think it is better to distinguish them as *nourishing cells*.

*Choreocolax* consists of a more or less hemispherical external portion made up of subdichotomously branched filaments enclosed and surrounded by gelatinous matter, and a mass of haustorial filaments growing within the host, ramifying at first in the cell-walls surrounding the central siphon, later spreading in all directions between the cells of the host, until the units of the haustorial filaments are almost as numerous as those of the external soma (Fig. 2). When these haustorial cells happen to reach the exterior of the host

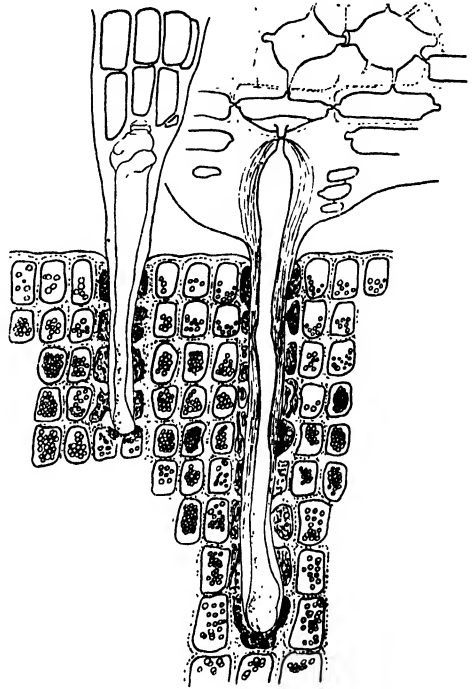


FIG. 3. Section showing haustoria (one young and one old) of *Polysiphonia* penetrating *Ascophyllum*. The black circles in the *Ascophyllum* cells represent the crimson physodes as shown when the fresh section is examined in vanillin and hydrochloric acid.

at a point sufficiently removed from that at which they originally entered, they frequently give rise to a second external soma. Such cases afford the only examples in which it is plain that the parasite does harm to the host, the multiplication of haustorial filaments often causing the host axis to break. The parasite becomes attached to the host where an opportunity can be found (Fig. 1), frequently in the axils of the host's branches, quite as often to the very young ramuli where antheridia are developing, and in fact to any place where a suitable opening occurs. The spores of the parasite are often emitted at low tide, so that almost the whole surface of the neighbouring *Polysiphonia* branches must be sprinkled by spores, and attachment is practically certain. I have never seen a *Poly-*

*siphonia* in the small bay in which *Choreocolax* occurs without several specimens of the parasite attached.

The somatic structure of *Choreocolax*, both external to and within the host, is very similar to that of *Harveyella pachyderma* and *H. mirabilis*. This is natural, for all are somatically reduced as far as possible, and the results in the case of small filamentous plants must be somewhat similar. The external soma of *Choreocolax* is more noticeably lobed than in either of the other two; these lobes are often seen in the mature cystocarpic plant, and it has been stated that the lobes are due to the presence of one cystocarp in each. But plants containing no cystocarps, and plants bearing tetrasporangia scattered evenly over the whole surface, are often equally lobed. These lobes are much more prominent in plants treated with alcohol, and when seen in the living plant are due to the greater development of one or more groups of peripheral filaments, probably owing to these clusters having accidentally received a better supply of food than others. After the first penetration of the host the early development of the external soma is very regular, and in vertical section it appears as a fan-like arrangement of subdichotomously branched filaments, the younger cells at the periphery being considerably smaller than the central units; all being more or less isodiametric in shape. But as the plant grows the difference between the peripheral layer and the remainder becomes very marked, the peripheral cells remain spherical or oval in shape, and only rarely is the length more than twice the breadth, while the central cells often become much elongated and set up the usual secondary connexions. Occasionally the central cells continue to be regularly arranged, and extreme differences in the shape and arrangement of these cells are seen in different plants. Figs. 4 and 15 show typical instances of the two extremes. As more filaments enter the host, and develop, they often displace cells of the pericentral siphons, and these become carried up by the growth of the parasite's external soma (Fig. 2, *d*), sometimes remaining unaltered for a very long time, for *Choreocolax* seldom directly attacks the main cell-contents of its host; the haustorial filaments ramify in the host cell-walls, and only show attachment to the contents of the cells at their pit-connexions. In old plants the cell-walls of the host do eventually break down after the cell-contents have disappeared. *Harveyella mirabilis* is the only Algal parasite in which I have seen such haustorial filaments actually break through the cell-walls of the host and grow freely inside, absorbing the cell-contents.

The outer membrane surrounding the external soma in *Choreocolax* is very thin as compared with that of *Harveyella*, and is plainly chiefly composed of the fused walls of the peripheral filaments. In *H. pachyderma* this is also at first the case, but the production of gelatinous material is so great that this outer membrane becomes homogeneous. Hence the two plants differ in external appearance under a lens; *H. pachyderma* has



a smooth surface in which the distal cells of the peripheral ramuli appear as evenly spaced units, in *Choreocolax* the surface is irregular, with the distal cells arranged in groups. The somatic reduction in *Choreocolax* has not yet entirely obliterated the original free separate thallus filaments.

The procarp commences as the distal cell of a peripheral ramulus and develops in the ordinary way. The young carpogonium is noticeably larger than the peripheral units surrounding it (Fig. 4, *a*, *b*, *c*). The trichogyne at first keeps pace with the growth in length of the peripheral ramuli, but soon passes beyond them, growing through the gelatinous outer membrane until it reaches the external medium. The carpogonium sends down a basal prolongation and three small cells are segmented, the carpogonial branch invariably consisting of three cells and a trichogyne. The length of the carpogonium with its accessory trichogyne, as in *H. pachyderma*, averages about 110  $\mu$ , but owing to the comparatively thin outer membrane through which the trichogyne has to pass in *Choreocolax*, it often projects into the water for 60  $\mu$  instead of for only 10 or 12  $\mu$ . as in *Harveyella*. Healthy mature procarys, existing at the same time in one *Choreocolax*, average from

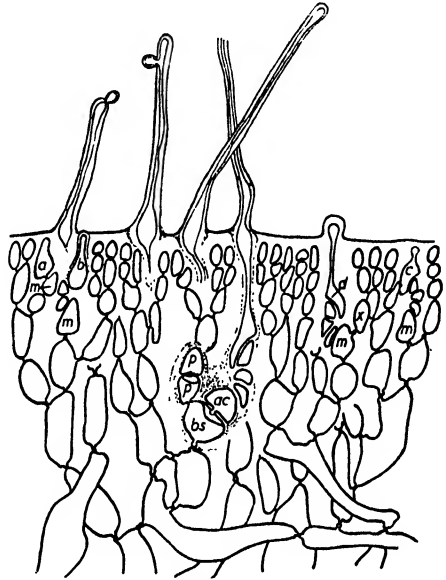


FIG. 4. *Choreocolax Polysiphoniae*. Development of procarp. *a*, *b*, *c*, *d*, young procarys; *m*, auxiliary mother-cell; *ac*, auxiliary cell; *bs*, basal cell; *pp*, filament developed from basal cell; *x*, first cell of similar filament developing from auxiliary mother-cell.

40 to 100 in number, as compared with an average of nearly 2,000 on a *Harveyella* of the same size. These procarys are usually distributed evenly through the peripheral layer, and, as they become old and buried in the soma, new ones are developed. Occasionally a number of procarys are found close together. I have seen as many as thirty trichogynes projecting from the surface of a living plant, almost touching each other. On sectioning this plant the procarys were found to be especially crowded, but not arranged definitely in any special manner. The remainder of the surface of this plant only showed seven projecting trichogynes. Although the number of procarys is comparatively small, the chance of fertilization is high. In the case of *Harveyella*, always submerged even at low tide, the clouds of spermatia may easily be lost in the volume of water, taking into consideration the comparative scarcity of the plant. But when a spermatium does by chance touch a suitable *Harveyella* it cannot avoid

the trichogynes crowded over the whole surface, and the product of one fertilized procarp occupies the whole available space in the soma. In *Choreocolax*, exposed to air for the greater part of its life, and with numerous plants situated on the closely crowded, down-hanging branches of *Polysiphonia*, contact between spermatium and trichogyne in the thin surrounding film of water is almost a certainty. The number of cystocarps produced in *Choreocolax* is usually from 5 to 8.

In *Choreocolax* the peripheral cell supporting the procarp is the auxiliary mother-cell (Fig. 4, *m*), which divides into an auxiliary cell and a basal cell after the trichogyne has reached the surface (Fig. 4, *ac*, *bs*). It is possible that this division of the auxiliary mother-cell takes place after fertilization, but I am only prepared to state definitely that it takes place after the trichogyne has reached the exterior, and never before. The basal cell gives rise to a short ramulus of apparently ordinary peripheral cells, growing towards the surface (Fig. 4, *p p*). Occasionally this little ramulus is begun before division of the auxiliary mother-cell (Fig. 4, *x*), and then is in appearance an ordinary peripheral ramulus growing upward from the subtending cell of the procarp. When the subtending cell does afterwards divide this little ramulus is always left attached to the basal cell, and then corresponds in appearance and in its future development with the one produced after division of the auxiliary mother-cell. This ramulus of, at the most, three or four cells may be compared to the two little sterile ramuli developed before division from the corresponding cell of *Harveyella mirabilis* (2, 10), but in the latter they are always developed basipetally towards the central part of the soma, and are at first noticeably smaller than the cells surrounding them; while in *Choreocolax* the single ramulus always grows upward and at first resembles in size and appearance the ordinary peripheral cells. In *Choreocolax*, should no zygote nucleus reach the auxiliary cell, this ramulus develops normally and soon becomes indistinguishable from the adjacent somatic cells.

After fertilization the zygote nucleus is transferred to the auxiliary cell by a very short *primary ooblastema*, the base of the carpogonium being at this time in close proximity to the auxiliary cell. Development takes place rapidly in some stages, with apparently one period of rest before maturity of the cystocarp is reached. This rest is at about the stage shown in Fig. 7. The auxiliary cell cuts off a shoulder by the usual obliquely curved wall, forming the first cell of the carposporophyte (Fig. 5, *g*).

At the same time the little ramulus (Fig. 4, *p p*) attached to the basal cell enlarges its cell-connexions (Fig. 5, *p p p* and *bs*). The carposporophyte soon becomes a small tuft of cells, while the filament, *p p*, develops into a curved coenocytic unit with the former basal cell as its lower part (Fig. 6). Frequently at this stage one or more cells may be cut off from any part of this coenocyte (Fig. 7, *cc*). Those from the upper part may either become

irregular projections (Fig. 8, *cc*) or groups of a few small cells; while the one from the former basal cell may set up a connexion with any neighbouring somatic cell, become a long irregular projection, or develop into

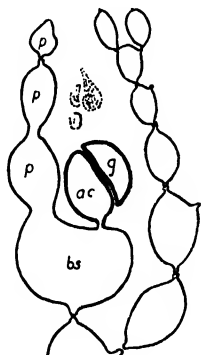


FIG. 5. Development of auxiliary cell. *g*, first gonimoblast cell; *ac*, auxiliary cell; *bs*, basal cell; *p p p*, coenocyte. The outlines of the cells of the carposporophyte in Figs. 5, 6, 7, 8, 9, 10, are thickened.

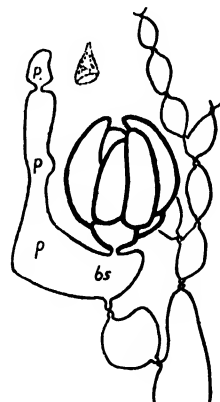


FIG. 6. Very young stage of carposporophyte. *bs*, basal cell; *p p p*, coenocyte.

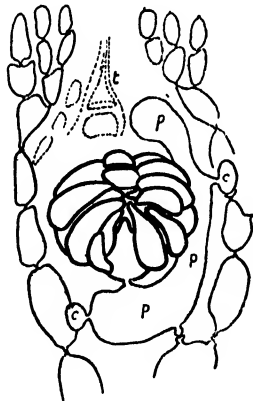


FIG. 7. Complete young carposporophyte. *p p p c c*, further development of coenocyte; *c*, relic of procarp.

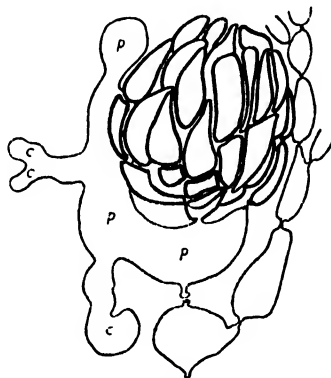


FIG. 8. Later stage in carposporophyte and coenocyte. *p* and *c*, coenocyte.

a filament of ordinary cells growing just outside the developing carposporophyte.

Up to this stage the secondary connexions, so common between the carposporophyte and the somatic cells, are absent. The young carposporophyte continues to grow until it forms a tuft of cells which passes beyond the coenocyte, *p p p*, and in many cases eventually surrounds it (Figs. 8, 9, 10).

Figs. 5, 6, 7, and 8 are complete young carposporophytes dissected out from the living plant, Figs. 9 and 10 are sections of carposporophytes in a similar stage of growth.

The carposporophyte thallus continues to grow until it forms a more

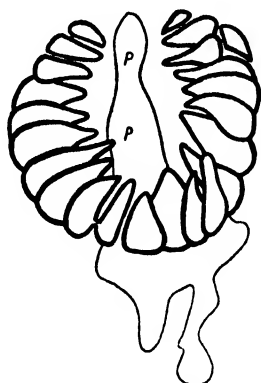


FIG. 9. Section of young carposporophyte.  
pp, coenocyte.

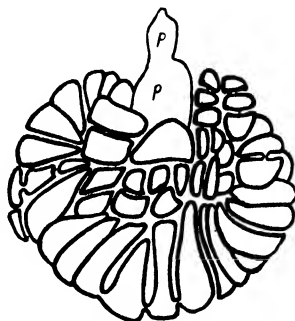


FIG. 10. Section of young carposporophyte.  
pp, coenocyte.

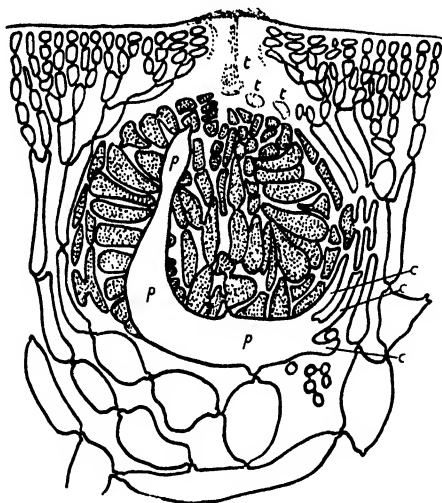


FIG. 11. Vertical section of half-mature carposporophyte. pp, coenocyte; ccc, projections from coenocyte; carposporophyte cells are dotted; ttt, relics of procarp.

or less flattened spherical mass of closely packed cells, of which Fig. 11 is a vertical section nearer to the circumference than to the centre of the sphere.

That the coenocyte, pp, is so prominent in this section is mere chance, as although it is always present at this stage and in the state shown in the figure, it is nearly always damaged by the section. Secondary con-

nexions between the coenocytic unit and the neighbouring cells are general, and the projections, *ccc*, are frequently the commencement of filaments growing around the carposporophyte. Near the apex in Fig. 11 the disintegrating remains of the trichogyne and two of the carpogonial branch units may be traced, and the ostiole is forming near the point at which the trichogyne pierced the gelatinous outer membrane of the soma.

I have not been able to make out the exact course of development in the ostiolar mechanism. At the apex of the carposporophyte a circular opening is plainly visible, for the filaments developing on its circumference do not meet at the summit of the sphere. Various small cells are developed at the circumference of this opening from the apical cells of the carposporophyte, and a little group of small units is often developed from the apex of the coenocyte; these small cells are in all about twenty in number, and resemble ordinary young peripheral units, but are smaller.

The peripheral cells of the soma, which are situated above the circular open apex of the carposporophyte, are naturally derived from and connected with the somatic filaments, united to it by secondary pits. Deterioration of the gelatinous material of the outer membrane of the plant to a readily stained mucilaginous condition soon occurs around the position formerly occupied by the trichogyne, and this disintegration proceeds downward towards the centre of the carposporophyte, involving a few of the original peripheral cells, and also at least the small cells produced from the apex of the coenocyte, but not, apparently, the small cells produced by the carposporophyte itself. The ostiole suddenly appears complete as a funnel-shaped opening to the exterior, and with a well-marked rim; a surface view showing it as a circular aperture, the peripheral thallus units situated nearest to the opening being compressed and often very irregular in shape.

A section of the completed ostiole is shown in Fig. 12. The young carposporophyte (Fig. 11) consists of a mass of somatic carposporophyte tissue, without as yet any carposporangia. By its growth the cells of the filaments surrounding it are pressed outwards, and these cells show considerable increase in length, in order to keep up with the growth. A certain amount of secondary connexion between the cells at the base of the carposporophyte, some of which are derived from the auxiliary cell, while others are somatic cells, takes place, and from the irregular fusion masses thus formed other elongated filaments arise growing round the carposporophyte among the already closely pressed somatic filaments, with which they make secondary connexions, forming a complicated reticulum of frequently connected filaments around the whole circumference of the carposporophyte except at its apex. Between this surrounding reticulum of long narrow cells, and the circumferential cells of the carposporophyte, as would be expected, connecting secondary pits are also established (Fig. 12).

This figure (12) is a section of a mature carposporophyte. The exterior

consists of a reticulum of interlacing filaments of elongated irregular cells, forming a hollow spherical fusion zone, on the inner surface of which are developed small tufts of short gonimoblasts, bearing carposporangia. The fusion-layer formation varies in its details, but the constituent filaments can usually be readily separated, those which have secondary connexions with the carposporophyte tissue being comparatively few in number; while

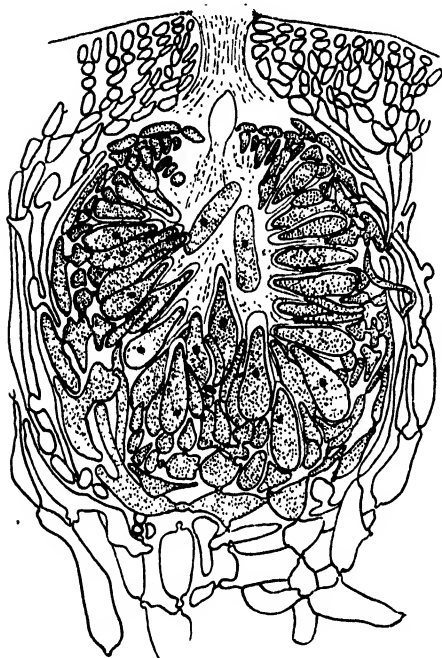


FIG. 12. Section of a mature carposporophyte. The carposporophyte cells are dotted.

many of the addressed investing somatic filaments remain wholly separate. When thus freed from the surrounding cells the fusion zone appears as a very irregular loose network, partly with primary or secondary connexions still intact, and partly fused into coenocytic aggregates (Fig. 13). The latter effect is almost continuous near the base, and often produces on the outside of the sphere small tufts of cells (Fig. 12), especially near the base and apex. As was mentioned before, these closely resemble small peripheral units.

The fusion zone produces tufts of short gonimoblasts. The position of these shown in Fig. 13 is misleading, owing to the pressure of the cover-glass on the preparation. The tufts of short ramuli are really developed periclinally, and spread over the surface of the sphere,

and on them are produced one or two celled ramuli bearing the carposporangia, and invariably directed towards the centre of the sphere. The whole structure is now a spherical mass of closely compacted cells in several layers. The outer zone is a tract of long, narrow, closely pressed somatic cells, not organically connected with the actual carposporophyte, and not specially developed as a cystocarp wall, but merely ordinary somatic filaments forced into this position by the growth of the carposporophyte. Among them are a few filaments belonging to the fusion mass at the base of the sphere, and between the various filaments secondary connexions do occur in consequence of such close proximity, but of no special theoretical significance. The next layer, part of the actual carposporophyte, consists of the very loosely arranged reticulum of flattened irregular cells (Fig. 13) more or less fused together, and giving rise laterally to tufts of short gonimoblasts.

These in their turn bear the carposporangia, which, developing centripetally towards the centre of the construction, force out the circumference until, when mature, it is subspherical. The development of these outer zones of filaments in the carposporophyte varies considerably in different individuals. No two are exactly alike in the amount of secondary connexion, the development of these connexions into complete fusions, or even the origin of the various elongated filaments. The variation between individuals closely resembling each other may be very minute, but the difference between the extremes is remarkable.

The carposporangia arise in pairs from a more or less pyramidal basal

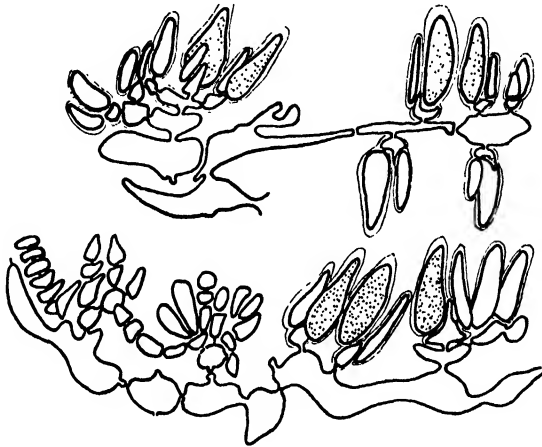


FIG. 13. Part of fusion zone, with gonimoblast filaments and carposporangia. The dotted units are carposporangia approaching maturity.

cell (Figs. 12 and 13) and are not developed simultaneously, the older of the pair being mature and ready to emit its carpospore when the second is still a slender unit. This arrangement has given rise to the statement that the carposporangia are intermixed with paraphyses, these latter being either very young carposporangia, or possibly walls from which the carpospores have been discharged. A small central portion of the carposporophyte is occupied by readily stained mucilaginous matter, the degenerated product of the former gelatinous intercellular material, and this continues up to the ostiole. The carposporangium wall ruptures at its apex, through which the carpospore is emitted (Fig. 12). In this figure can be seen the last degenerating relic of the original coenocyte derived from the basal cell, and in previous figures marked *ppp*.

The development and eventual disappearance of this coenocytic unit may now be considered. One advantage of a laterally inserted carpogonial ramulus, as compared with the more primitive terminal position, is that the carpogonium or young carposporophyte in the former case is able to drain

food material from the filament units above the subtending cell of the carpogonial ramulus. As the carpogonium becomes buried more deeply in the soma and thus is farther removed from the light, so its carposporophyte will be able to drain food from an increasing number of young cells. In *Choreocolax*, after the development of the carpogonial ramulus, the continuation of the main filament is delayed, or often limited for a time to one cell (Fig. 4, *b* and *c*, and *d*, *x*), but at about the time the trichogyne has reached maturity the development is resumed (Fig. 4, *pp*). This is not a development from the auxiliary cell, but from the original somatic sub-

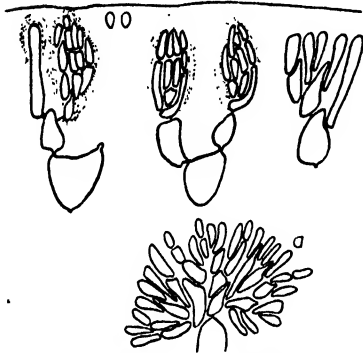


FIG. 14. Antheridia of *Choreocolax*. *a*, small tuft pressed out.

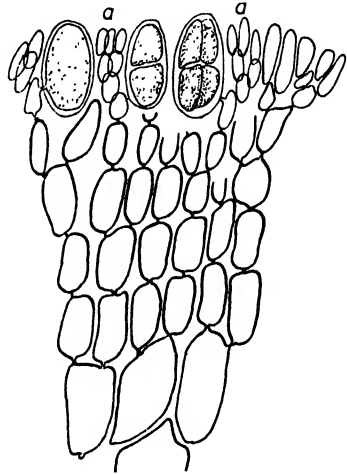


FIG. 15. Tetrasporangia (dotted).

tending cell, now the basal cell. That the units of such a filament, whether normal or delayed, should enlarge their connexions and give rise to an irregular coenocyte is quite usual (Figs. 5, 6, 7, 8, *ppp*), in order to facilitate the drainage of the food contents to the rapidly growing young carposporophyte. I do not consider that this noticeable coenocytic structure in *Choreocolax* has any other significance; it represents the distal units of the normal filament of which the auxiliary mother-cell is one unit, for should the auxiliary cell not receive any zygote nucleus, the cells of this filament develop as normal peripheral cells, and do not become coenocytic.

The antheridia more resemble those of *H. mirabilis* than those of *H. pachyderma*. They are produced in very distinct clusters, while in *H. pachyderma* the production is so even that the separate groups can scarcely be distinguished. The final result is the same, a complete zone of spermatia surrounding the whole external soma. At first the longitudinal divisions are very rapid, and the effect of the tightly packed cluster (Fig. 14) is very characteristic of *Choreocolax*; when, later, the constituent cells of



the ramuli spread out they are almost indistinguishable from those of *H. mirabilis*.

The tetrasporangia (Fig. 15) differ from those of *Harveyella* only in the ramulus bearing them. When mature the tuft in *Harveyella* consists of the tetrasporangium and one or two units in addition; in *Choreocolax* the additional units number five or six (Fig. 15, a). Thus the tetrasporangia are not set so closely together. The development and emission of the tetraspores differ in no way from *Harveyella*.

Undoubtedly of the three parasites now examined, *Choreocolax* is the most successful in that it is more abundant, and is not affected in numbers or habitat by the variations of temperature or light such as occur at Plymouth. In this locality its distribution appears to be only controlled by the occurrence of its host.

Owing to its small size and brown colour it is difficult to see with the naked eye, and this probably accounts for my not having found it on the southern coast of Ireland, and also possibly for the lack of definite information as to the time when it is abundant in other places.

In my paper 'On the Life-history of *Harveyella pachyderma* and *H. mirabilis*' (2) it was shown that these two plants are sufficiently distinct to be placed in different genera. The renaming of *H. pachyderma* was postponed until the genus *Choreocolax* had been investigated, and I now suggest that the name *Holmsella pachyderma* should be substituted for the erroneous name *Harveyella pachyderma*. *Harveyella mirabilis* will still remain as the typical species of the genus *Harveyella*, and *Choreocolax Polysiphoniae* as the typical species of the genus *Choreocolax*. The distinguishing characters of the three genera are given later in this paper.

These three parasitic Florideae, *Choreocolax*, *Harveyella*, and *Holmsella*, are obviously little strays of three groups which have managed to live on owing to their success as parasites. Beginning as Florideae with a fully elaborated reproductive cycle, they, in spite of probably very inferior somatic equipment, have saved themselves from extinction in the struggle for substratum when other closely allied genera and species may have perished, and are so far eminently convergent in biology and equipment. Hence it would not be surprising if these parasites should not be found to fit very well into the present scheme of Floridean classification, based, as this has been, on the auxiliary-cell attainment of a large number of genera, the majority of which have not been studied in detail. Adopting the modification of the classification of Schmitz, given by Kylin (11), and putting on one side the haplobiontic and diplobiontic division suggested by Svedelius as not yet workable, the Florideae can be divided into two groups.

A. The fertilized carpogonium itself gives rise directly to the gonimo-

blast system of the carposporophyte. These gonimoblasts may or may not unite with food cells. This group includes Nemalionales and Gelidiales.

- B. True auxiliary cells serve as the starting-point for gonimoblast formation. This group includes Cryptonemiales, Gigartinales, Rhodomeniales, and Ceramiales.

The three small parasites all possess definite auxiliary cells from which gonimoblast formation is initiated. *Harveyella mirabilis*, with its auxiliary cell undoubtedly segmented after fertilization, is definitely one of the Ceramiales. As Kylin (11) remarks, it agrees with the Rhodomelaceae in many respects, but in its structure are many characteristics which do not agree with those of that group. In fact it is not possible at present to decide to which division of the Florideae *Harveyella* belongs. Similar remarks might be applied to *Holmsella* and *Choreocolax*, which by their auxiliary-cell formation undoubtedly belong to Group 'B', but are difficult to include. Now all three have carposporophytes, which, when mature, resemble very remarkably those of certain members of the Nemalioninae in Group 'A'. *Harveyella* and *Holmsella* have carposporophytes when mature of the *Dermonema* type, while that of *Choreocolax* very closely resembles that of *Galaxaura*.

So that under the present classification the three small parasites, though definitely outside the Nemalioninae according to the early development of their carposporophytes, in the structure of these carposporophytes when mature closely resemble certain members of that group, and again do not much resemble those of *any other members of the Florideae*. It has been stated that they are aberrant members of the Rhodomelaceae or of the Gelidiaceae, with peculiarities due to parasitic deterioration. The parasitic habit does produce extreme somatic reduction, but not necessarily deterioration of the reproductive mechanism; and although somatically reduced to a very great extent, the carposporophytes of all three parasites are still fully Floridean, and are in fact less degenerate than those of many non-parasitic Florideae. In *Harveyella mirabilis* there is no fusion of sporophytic and somatic cells, except the one connexion at the auxiliary cell; in *Choreocolax* a certain number of fusions take place, but they are not numerous, and in *Holmsella* a zone of fusion cells is produced as a more or less horizontal layer throughout the external soma; but even in this case, where the parasitism of the sporophyte has proceeded farthest, it is still possible to make out the origin of each constituent of the fusion zone.

Unfortunately, there are no detailed accounts of the development of the carposporophyte in *Galaxaura* and *Dermonema*, with which the development in the three parasites could be compared.

As *Choreocolax*, *Harveyella*, and *Holmsella* possess mature carposporo-

phytes so closely resembling in structure those of two of the Nemalioninae, it is necessary to examine this group more in detail before deciding on the position to which the three parasites should be assigned.

**Nemalioninae** (Schmitz, 1889), **Nemalionales** (Schmitz and Hauptfleisch, 1896).

A number of more or less elementary types of the Florideae have been included in this group solely from the point of view that the carposporophyte arises directly<sup>1</sup> from the carpogonium and not from an auxiliary cell. The group may be regarded as a collection of relics of various phyla, the chief interest of which lies in the traces of primitive phases of the development of the Florideae which many of them show. Unfortunately, details of the development of many are so scanty that the group can only be regarded, at present, as a convenient convention.

The Nemalioninae can be conveniently divided into two main sections:

1. The Nemalionales, comprising the genera *Nemalion*, *Helminthocladia*, *Helminthora*, *Batrochospermum*, *Chantransia*, *Lemanea*, *Scinaia*, &c. In this section the carposporophyte has no haustorial connexion with true auxiliary cells or with nourishing cells; the radially organized carposporophyte is at its simplest.

2. The Gelidiales, including *Dermonema*, *Galaxaura*, *Chaetangium*, *Wrangelia*, *Naccaria*, and *Gelidium*. In these the carposporophyte in many cases has numerous haustorial connexions with nourishing cells, but not with true auxiliary cells. They possess generally a more specialized dorsiventral carposporophyte. In some cases the members of this section possess tetrasporangia, and probably the full cycle of the life-history. It is difficult to draw a definite boundary line between the more advanced forms of this section and the Gigartinales, into which they appear to grade.

The various types in the two sections show a considerable range in the construction of the thallus, though in none is it of any great size, varying from a bushy growth of three feet sometimes in *Helminthocladia*, to a height of less than a millimetre in some *Chantransias*. The thallus may be of the more primitive cable-strand type without apical cell, as in *Nemalion*, *Helminthocladia*, or *Dermonema*, or of the same type, calcified, as in *Galaxaura*, some species of which approach the organization of *Corallina*. Many genera possess a thallus of the more advanced axial filament type, corticated in *Lemanea* and *Gelidium*.

Similarly in the reproductive details considerable variations are found.

The tetrasporophyte in *Nemalion* and *Scinaia* appears to be entirely suppressed, the meiotic division being established at another point, while in several other genera tetraspores are wanting or not yet described.

The carpogonial branch may be of the primitive erect terminal type,

as in *Nemalion*, *Batrochospermum*, *Chantransia*, and *Lemanea*, or of the more advanced laterally inserted type, as in *Helminthocladia*, *Dermonema*, &c.

While no section possesses either all the more primitive or all the more advanced characters, yet the members of the Gelidiales show an all-round improvement; the section Nemalionales includes in types like *Trichogloea* the greatest number of primitive characters, while *Gelidium*, of the axial filament type, corticated, with a bilateral frond system, extensive haustorial connexions with nourishing cells, and special conceptacles with ostioles, is perhaps the most highly organized.

Although *Harveyella*, *Choreocolax*, and *Holmsella* are very simple in thallus structure, and must, even before they became parasitic, have possessed a somatic organization of the cable-strand type, with no apical cell; yet in the characters of their carposporophytes they are not elementary. They have dorsiventral carposporophytes, all having haustorial connexions with true auxiliary cells. It is, of course, possible that further research may discover true auxiliary cells in some of the genera now included in the Gelidiales, and this would necessitate their removal from that group; for while the cytology of a Floridean may be decadent, and the want of tetraspores due to the same cause, yet if once the carposporophyte has become parasitic on an auxiliary cell, it will never let go. The early Florideae may have had no need for an auxiliary cell, and possibly did not possess one. The group Nemalioninae, founded on the absence of true auxiliary cells, is a good one, and however much *Choreocolax*, *Harveyella*, and *Holmsella* may in their mature carposporophytes resemble *Galaxaura* and *Dermonema*, their possession of auxiliary cells makes their inclusion in either section of the Nemalioninae impossible. As their mature carposporophytes do not resemble those of any other group of the Florideae, I consider that the three parasitic genera should be retained as a separate group until the detailed development of many more Floridean genera is known. They might be provisionally arranged and defined as follows:

**Choreocolaceae**, a new provisional family (which may be placed for the present in the also provisional group Gigartinales).

Holoparasitic Florideae, without chlorophyll. Somatic organization of the cable-strand type, very much reduced. The soma consists of a larger portion external to the host, made up of branched filaments enclosed in a gelatinous membrane, forming a subspherical flattened cushion; the average diameters of this external thallus are: height 0.1 to 0.7 mm., length 0.8 mm. The smaller portion of the thallus consists of much-branched haustorial filaments ramifying among the tissues of the host. Carpogonial ramuli laterally inserted. Carposporophyte dorsiventral, arising from a true auxiliary cell.

Genus 1. *Choreocolax*, Reinsch.

Auxiliary cell cut off from the subtending cell of the carpogonial ramulus. Carposporophyte subspherical, consisting of an outer close reticulum of elongated cells, from which are developed in the enclosed cavity tufts of short gonimoblasts bearing carposporangia; the whole is surrounded by the closely pressed filaments of the vegetative thallus, but there is no specially developed carposporophyte wall. Opens to the exterior by an ostiole.

Species 1. *Choreocolax Polysiphoniae*, Reinsch.

Parasitic on *Polysiphonia fastigiata*. External soma surrounded by a thin membrane. Carpogonial ramulus consists of three cells and a trichogyne. Antheridia lining the whole surface of the external soma in distinct tufts.

Species 2. *Choreocolax tumidus*, Reinsch. Parasitic on *Ceramium involutum*.

Species 3. *Choreocolax Cystoclonii*, Reinsch. Parasitic on *Cystoclonium purpurascens*.

Species 2 and 3 are doubtful, as nothing is known of the development of the carposporophyte.

Genus 2. *Harveyella*, Schmitz and Reinke.

The auxiliary cell is cut off from the subtending cell of the procarp, after fertilization. Carposporophyte consists of a more or less horizontal layer of free elongated filaments, without further secondary connexion with the cells of the vegetative thallus. From this ooblastema vertical tufts of gonimoblasts arise bearing carposporangia in the space formed by the elongation of the basal cells of the periphery.

Species. *Harveyella mirabilis*, Schmitz and Reinke.

Parasitic on *Rhodomela subfusca*. External soma surrounded by a thin membrane. Haustorial ramifications very largely developed between and in the cells of the host. Carpogonial ramulus three cells and a trichogyne. The auxiliary mother-cell produces from its basal part two small ramuli of two and four cells respectively before fertilization.

Genus 3. *Holmsella*, Sturch, nov. gen.

The auxiliary cell is not formed from the subtending cell of the carpogonial ramulus. The carposporophyte consists of a more or less horizontal layer of long filaments, and this ooblastema unites by secondary connexions with every cell of the vegetative thallus immediately beneath it, forming an irregular fusion zone from which gonimoblasts arise in vertical tufts, bearing carposporangia in the space formed by the elongation of the basal cells of the periphery.

Species. *Holmsella pachyderma*, Sturch, nov. comb. (formerly *Harveyella pachyderma*, Holmes and Batters).

Parasitic on *Gracilaria confervoides*. External soma surrounded by a very thick membrane. Carpogonial ramulus one cell and a trichogyne.

The drawings were all made from fresh plants. As the figures have been enlarged and afterwards reduced, instead of stating the original magnifications I have appended the actual measurements of various parts.

Average diameters of external soma, height 0.3 mm., width 0.6 mm.

" " carposporophyte, 210  $\mu$  and 200  $\mu$ .

" length of ostiole from apex of carposporophyte to exterior, 60  $\mu$ .

" width of ostiole, from 14  $\mu$  to 35  $\mu$ .

" dimensions of carposporangia, length 40  $\mu$ , width 10  $\mu$  to 13  $\mu$ .

" " young carposporangia, growing on the same basal cells as the above mature ones, 25  $\mu$  by 4  $\mu$ .

" dimensions of free carpospores before emission through the ostiole, 37  $\mu$  by 11  $\mu$ .

" dimensions of tetrasporangium, length 60  $\mu$ , width 30  $\mu$ .

" " tetraspore, length 28  $\mu$ , width 14  $\mu$  to 20  $\mu$  before emission.

In conclusion, my thanks are especially due to Dr. A. H. Church, M.A., of Oxford, for very valuable assistance in the preparation of this paper.

#### SUMMARY.

*Choreocolax* is holoparasitic on *Polysiphonia fastigiata*, itself hemiparasitic on *Ascophyllum nodosum*.

The subtending cell of the procarp functions as the auxiliary mother-cell, and after the transference of the zygote nucleus the carposporophyte is developed from the auxiliary cell.

All the stages of the development of the carposporophyte are described in detail, the mature cystocarp much resembling that of *Galaxaura*. As the three plants, *Choreocolax Polysiphoniae*, *Harveyella mirabilis*, and *Harveyella pachyderma*, differ wholly from each other in the development of the carposporophyte, the name *Holmsella pachyderma* is suggested in the place of the erroneous name *Harveyella pachyderma*.

It is shown that these three parasitic genera cannot be included in the group *Nemalioninae* of Schmitz, and, as their mature carposporophytes do not resemble those of any other Floridean group, it is suggested that they should remain for the present unattached as a small group of parasitic Florideae.

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# The Development of the Perithecium of *Ophiobolus graminis*, Sacc.

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With Plates XVIII and XIX and eight Figures in the Text.

## INTRODUCTION.

THE 'Take-all' or 'White-head' disease of cereals attributed to the fungus *Ophiobolus graminis* (a minute member of the Pyrenomycetes) has received considerable attention from an economic standpoint. Interesting papers bearing upon this aspect of the disease have been written by Delacroix (8), Prillieux (27), M<sup>c</sup>Alpine (22), Mangin (23, 24), Richardson (28), Robinson (29), Waters (34), Kirby (19), and others. Kirby's recent excellent memoir gives an extensive account of the history, ecology, and control of the malady.

The disease has recently made its appearance in Wales on oats, and as the above-named writers have described its occurrence largely on wheat, declaring oats to all appearances to be immune, it may be of interest to give here a brief account of the symptoms on this host. When the oats are in flower, the affected plants can readily be detected in the field because they stand out as white or bleached areas amongst the unaffected green plants. An appearance is presented as if parts of the crop had prematurely ripened, but there is no dwarfing of the haulms as described by Kirby for wheat. When such plants are examined it is found that the glumes are empty of grain, and though the haulms are rigid and erect the plants are really dead down to the roots. As soon as the bleached condition becomes apparent, the fungoid growth can be recognized on the roots and on the basal internode of the culm, and if the protective leaf-sheath in this region be drawn away, a coarse mat or web composed of a dark-brown chitinous mycelium is seen to clothe the haulm and inner surface of the sheath. The

perithecia occur in large numbers early in August, and they may appear singly or in small groups. It is a striking feature that they do not grow on the superficial web but arise endogenously from the mycelium which is within the leaf-sheath, at the inner surface of which their curved beaks will eventually break through. On rare occasions the perithecia are also found on the roots.

#### METHODS.

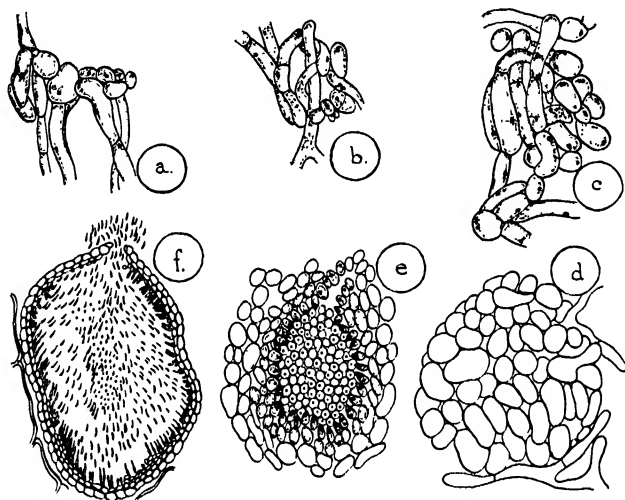
The present investigation was carried out entirely by means of microtome sections of field material, and no attempts at artificial cultures were made. The material was fixed in Flemming's strong mixture diluted with an equal volume of water, and the sections were stained with Heidenhain's iron-alum-haematoxylin, followed by a counterstain of 1 per cent. Congo red in water.

#### THE MYCELIUM.

There are two types of vegetative mycelium in this fungus. One kind consists of delicate hyphae of narrow diameter, the walls and contents of which stain sharply, and the other of hyphae of greater width and longer cells in which the walls are dark brown and chitinous in appearance. Whereas the narrow hyphae consist of cells of uniform diameter, the dark hyphae in addition are frequently seen to contain very large oval-shaped cells. The cells of the brown hyphae are not so rich in protoplasmic contents, and their walls remain untouched by the counterstain. In both kinds of hyphae, however, the cells are uninucleate and guttulate, but numerous cases may be found of cells having two or three nuclei. Closer examination reveals the fact that the one kind of mycelium gives rise to the other; the delicate hyphae can be seen in places to become wider, and this increase in diameter is accompanied by peculiar oblique striations in their walls, but which later become obscured by the appearance of the brown colour. The mycelium shows a fair amount of branching and is intracellular. It can, however, by penetration of the cell-walls reach the exterior of the host to form the dark parenchymatous webs already mentioned. Within the host-cells the hyphae become variously twisted and densely coiled, but when they appear in the elongated elements of the wood and bast, the individual threads may be seen to attain considerable length without branching. Within the narrow confines of these elements of the host, the hyphal filaments may become twisted and anastomosed to resemble rhizomorphic strands. The nuclei in the vegetative cells of *Ophiobolus* are very small, and under high magnification appear only as small homogeneously staining bodies. There is no evidence of a nuclear cavity or reticulum, and on account of the minute size of the nuclei it is impossible to detect stages of division.

## THE SPERMOGONIA.

When the material collected from the field in August was examined, it was found that the epidermal cells of the leaf-sheath were torn in an irregular manner. At this time there are found in these ruptured epidermal cells small round masses of closely interwoven hyphae consisting of the delicate mycelium mentioned above (Text-fig. 1, *d*). At maturity they are seen in optical section to be spherical or ovoid in shape, with a wall three to six cells in thickness, and at one point on the periphery a slight pro-



TEXT-FIG. 1. Development of the spermogonium. *a, b, c.* Early stages showing approximation and interweaving of hyphae to form a hollow sphere (*d*).  $\times 1,750$ . *e.* Median section of a young spermogonium; the interior is hollow and lined with pear-shaped cells which form the spermatial hyphae; ostiole at apex.  $\times 1,400$ . *f.* Median section of a mature spermogonium containing spermatia and spermatial hyphae on the internal wall.  $\times 500$ .

tubercance reveals the presence of an ostiole (Text-fig. 1, *f*). These receptacles are filled with dense masses of exceedingly minute cells which show a radiate arrangement from the wall to the interior, together with a convergence towards the ostiole, where they are often seen to collect in extruded masses. These bodies and their contents bear evident resemblance to spermogonia and their spermatia. These receptacles in the course of development follow the 'symphogenous' type of de Bary (9) and Kempton (18), the 'Knäuelfrucht' of Zopf (36), and the 'angiocarpous' of Higgins (16). At the initiation of a receptacle the tips of a number of hyphae congregate together, and by further growth and intertwining there is gradually formed a loosely woven ball of hyphae (Text-fig. 1, *a, b, c*). Further growth and interpolation of hyphae into the outer layers of the wall bring about a general expansion of the whole body, and by intimate coalescence of the

elements at the periphery the surface of the ball becomes distinctly parenchymatous in appearance (Text-fig. 1, *d*). The expansion of the body due to rapid growth of the wall results in the formation of a hollow sphere (Text-fig. 1, *e*), and the cavity so formed is bounded by cells, which in most parts appear as a single layer, but at other points they are seen to be in two or three layers. These cells lining the cavity stand out somewhat sharply from the outermost cells by their greater quantity of protoplasm and more prominent nuclei. They are pear-shaped, with their narrow ends inclining towards the centre of the cavity. These cells are invariably uninucleate. They give rise to short, narrow cells of varying length which in turn abstrict from their apices the diminutive cells mentioned above. In favourable preparations the latter are seen arranged in short chains. In nearly all the slides examined these small cells are seen scattered over the sections, and advantage was taken of this to examine them in detail. They appear as small cells of narrow diameter, blunted at one end and narrowing almost to a point at the other extremity. They measure from 5 to 7  $\mu$  by 1 to 1.5  $\mu$  and exhibit a very distinct curvature. A striking feature in them is the prominence of the nucleus, which no doubt is rendered so conspicuous by the almost complete absence of stainable contents around it. Their cytoplasm is very scanty and they seem to be quite devoid of food reserve. In addition, there are found interspersed among the spermatia long hyphae of extreme tenuity, which have their origin in the cells lining the cavity and which radiate from the wall to the ostiole. Such hyphae have been seen by Brooks (5) in the spermogonia of *Gnomonia*, and he is of opinion that they may assist in the extrusion of the spermatia. Kirby (19) reports the presence of minute sickle-shaped microconidia (measuring 5.5 to 8  $\mu$  by 1.5 to 2  $\mu$ ) which seemed to be functionless. Mangin (24) considered them sporidia, and M<sup>c</sup>Alpine (22) recorded their presence without giving any hint as to their function. From these considerations one is inclined to conclude that these cells, from their small size, the manner in which they are formed, and the time of their appearance, are probably homologous with spermatia. The significance of spermatia in the life-history of this fungus is discussed below in connexion with the structure of the ascogonia.

#### THE ARCHICARPS.

Early stages in the formation of the perithecia are found at about the same time as the appearance of the spermogonia. These stages are so strikingly different from those of spermogonial development that there is no possibility of confusion between them. Within some of the cells of the epidermis of the leaf-sheath, and frequently of the mesophyll as well, there are seen small coils (usually one coil to each cell) of hyphae which stain more deeply than those of the vegetative mycelium, but which otherwise

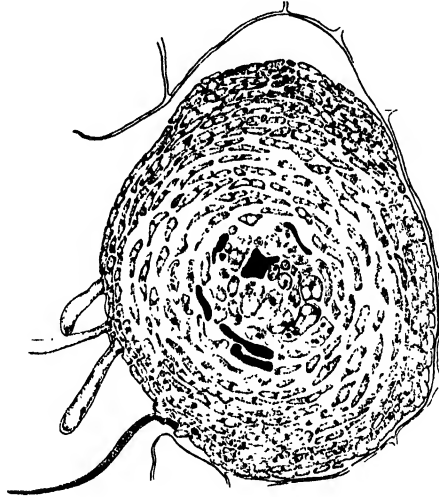
are very similar to them. In favourable preparations showing very early stages, when the coil is fairly loose, it appears to be formed from a single hypha (Pl. XVIII, Figs. 4, 5). It is very difficult to be certain on this point on account of the increased coiling of the hypha in its growth (Pl. XVIII, Figs. 2, 3), and also from the rapid encroachment of vegetative hyphae which will eventually invest it. The coil is divided into a number of uninucleate cells, and further stages show that any number of these cells may branch towards the interior of the coil, each to give rise to another cell. The new cells are cut off by a wall, and they are altogether larger and stain somewhat more deeply than the parent cells. They in turn produce near the apex single attenuated branches which curve over to lie parallel with them. The bent branches may or may not be separated from the cell by a cross-wall, but as the whole object is small and very retentive of stain, it is often very difficult to detect a septum between them (Pl. XVIII, Figs. 2, 3, 4, 5). The coil and its branches may now be interpreted as different parts of an archicarp. No cell or hypha comparable with an antheridium is to be seen at any stage. It must be stated at this point that further stages in the development of the perithecium of *Ophiobolus* will show that the archicarps are abortive structures. It may, however, be interesting to compare them with those of other members of the Pyrenomycetes. One is inclined to the view that the whole coil is a specialized hypha, each cell of which may give rise, in the manner described, to another of greater diameter and denser contents—the oogonium, with the parent cell as its stalk, while the prolongation of the oogonium into a curved beak may probably represent a trichogyne. A single nucleus exhibiting no more differentiation than that within a vegetative cell appears in each of these elements. This uninucleate condition has been observed by Baur (2) and Darbishire (7) in Lichen types, by Moreau (25) in *Peckiella*, Zopf (35) in *Chaetomium spirale*, Vallory (32) in *Chaetomium Kunzeanum*, Dangeard (6) in *Sordaria*, *Podospora*, and others. Though this condition is clear in the young archicarp, yet there are frequent evidences that the larger (oogonial) cells may later contain two or three nuclei. I have not been able to detect any signs of nuclear fusion in these cells. Further, if more than one cell in a coil will branch in the manner described, then a number of archicarps arise (Pl. XVIII, Fig. 3), and when these are eventually covered over by the encroaching and interlacing vegetative hyphae, it is evident that a number of ascogonia may become incorporated within a single perithecium (Pl. XVIII, Figs. 6, 7).

#### PERITHECIAL DEVELOPMENT.

The ascogonial coils appear in the preparations as heavily stained cells even up to the stages of ascus formation (Text-fig. 6). In sections of very young perithecia (Pl. XVIII, Figs. 6, 7) the dark cells stand out very prominently and in great contrast to the remaining tissues. These cells may often appear

to be isolated from each other, but when the sections are examined in series they can be seen to form parts of a coil. They do not appear at any time to become emptied of their contents, and they persist to such an extent that the tissues which later develop at the centre of the young perithecium push them aside towards the perithecial wall, where they often appear crushed or distorted. Despite careful search no evidence can be obtained that any part of these coils contributes to the formation of ascogenous hyphae.

One must now return to the stage at which a coil appears. In close proximity to it, a few hyphae of delicately stained vegetative mycelium are



TEXT-FIG. 2. Median longitudinal section of a young perithecium showing differentiation into core- and wall-tissues. The dark cells are those of ascogonial coils. The protuberance at the apex shows the seat of the future neck and ostiole.  $\times 1,050$ .

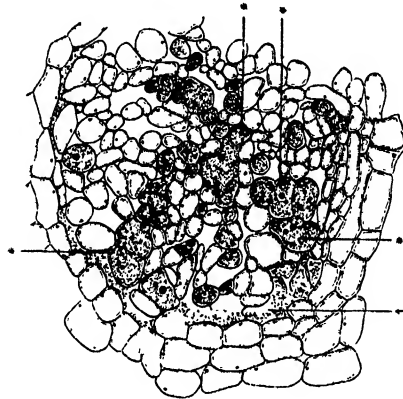
seen to grow towards the coil, and to insinuate their tips into the space bounded by it (Pl. XVIII, Figs. 4, 5). These hyphae are not to be confused with, say, broken parts of a coil, for not only are they frequently seen to be at right angles to the curve of the coil, but their staining reaction is also much more delicate, and, moreover, their nuclei are better defined than those seen in the coil. Immediately upon their entry into the space, the tips of the intruding hyphae become dilated, and the swollen terminations are then cut off by cross-walls. This process of the interpolation of hyphae seems to occur at various points at the periphery of the coil, so that it is only at a very early stage that the process can be distinguished from the investment of the coil by other vegetative hyphae, which by interweaving go to form the wall of the perithecium. Moreover, the investing filaments do not exhibit swollen tips, but otherwise they are not to be distinguished in general appearance from the intruding hyphae. With the penetration of

additional hyphae into the space of the coil, and possibly as the result of growth and division of the cells already pushed in, the young perithecium in median section is now seen to have a distinct core of oval-shaped cells (Text-fig. 2 and Pl. XVIII, Fig. 6). At the earliest stages in the formation of the perithecial investment, its cells are seen to be elongated in a tangential direction, and arranged as if in a spiral fashion in which the inner turnings—about six in number—are somewhat loosely but very regularly arranged, standing out rather sharply against the peripheral portion of the spiral in which the cells are closely packed together. At this stage it becomes evident that the deposition of the outer perithecial layers is not equally centrifugal, for at a part in the periphery of the perithecium the layers form a protuberance. This is the region in which the neck and ostiole will be developed (Text-fig. 2). Concurrently with the early formation of the perithecial walls certain changes of great importance occur in the tissues of the core. The dark-stained ascogonial coil situated at the periphery of the core is still very prominent (Text-fig. 2), and here may be recognized possibly one of the large oogonial cells, and in close proximity on its right a narrow strand jet black in colour, which is no doubt its trichogyne. The cells of the core at their earliest intrusion into the coil are seen to contain, some, one nucleus, whereas others may have two (Pl. XVIII, Figs. 4, 5), and in rare cases three or four nuclei may be seen. Whether the multinucleate condition is derived in the course of cell-formation from a parent hypha, or whether it occurs as the result of division of a primary nucleus, could not be determined. When the core-cells are seen to have increased considerably in number, a marked differentiation occurs in the density of their cytoplasm. As development proceeds this differentiation becomes still more apparent, to such an extent that the section in this region presents a distinctly mottled appearance (Text-fig. 3). This is due to the fact that the great majority of the core-cells are rapidly becoming so vacuolated that their cytoplasm is reduced to mere utricles. Since the core-cells at their initiation show no differentiation in shape or contents (Pl. XVIII, Fig. 6), there is no doubt that the greater density of contents in some of them is due to increased nourishment at the expense of the others. At this time the perithecium has increased somewhat in bulk, and it is now possible to detect around the core a definite boundary, consisting of a zone at some parts of one, and at others of two or three, of the innermost layers of the perithecial wall, which have become narrowed through the opposed pressure of the expanding tissues within and of those external to them.

Those cells of the core that possess dense contents are seen (in a median section of the perithecium) to be more numerous towards a third or a fourth part of the circumference of the limiting zone. With this arc as a base, these cells appear to radiate outwards from it into the core in the form of irregular chains (Text-fig. 3). The concave side of the arc in the zone is

situated diametrically opposite to the protuberance at the periphery of the perithecium, where the neck and the ostiole are later to appear.

The nuclei which are present in the dense cells of the core are now much more prominent than those within the vacuolated cells. Moreover, most of the dense cells are seen to contain at least a pair of nuclei, and it is somewhat rare to find any which are uninucleate. A striking feature is the inequality in size amongst these nuclei, and whilst none approach the diminutive size of those in the vacuolated cells, there exist all gradations from the smallest to the largest. A feature common to them all, however, is

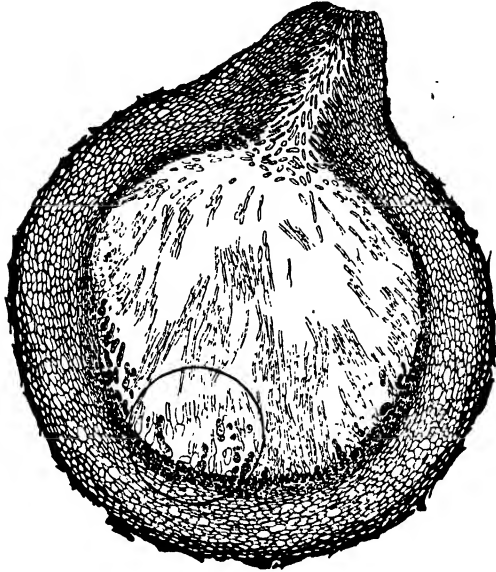


TEXT-FIG. 3. Portion of a median section of the core-tissue showing advanced differentiation of its cells into those with dense contents and others considerably vacuolated (the 'vacuolated parenchyma'). The asterisks indicate situations where conjugated cells occur amongst the dense cells. At the right hand, the inner layers of the wall have become narrow to form the limiting layers of the core. The arrow points to the position of these layers at the base of the core, and in them the granular change has begun. Note variation in size of the nuclei in the dense cells.  $\times 1,050$ .

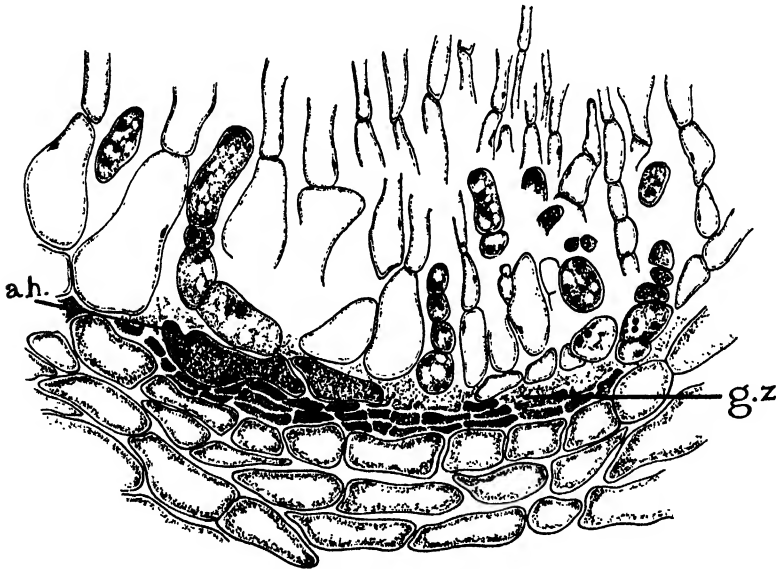
that they appear as dark homogeneously stained spheres, showing no signs of nuclear differentiation.

Whilst careful examination of a chain of the dense cells frequently shows that it is an easy matter to follow the contour of its constituent cells in optical section, against the abutting vacuolated parenchyma, it is frequently impossible to detect the cross-septa between these cells of a chain. A factor of great interest may, however, account for this difficulty. In favourable preparations it is seen that *the septa between two or more cells of a chain are perforated, thus giving rise to a compound cell*. This phenomenon of conjugation of cells is clearly seen at various points indicated in Text-fig. 3 and in Pl. XVIII, Fig. 8. It may be urged that such conjugated cells only represent stages of cell-growth in which the cross-walls have not yet appeared, as would occur, for instance, in the formation of ascogenous hyphae. Against this suggestion is the fact that at the early appearance of cell-conjugation (Text-fig. 3 and Pl. XVIII, Fig. 8) the vacuolated cells do not become crushed, as would be the case if the cells abutting on them showed





TEXT-FIG. 4. Median longitudinal section of a perithecium at time of formation of ascogenous hyphae and showing the mutual separation of the vacuolated parenchyma to form the paraphyses-like filaments. The limiting layers of the core are represented diagrammatically by the semicircular zone of black cells. The formation of periphyses is well advanced. The portion within the circle is shown on a larger scale in the next figure. (Drawn from a micro-photograph.)



TEXT-FIG. 5. The portion within the circle in Fig. 4. The limiting layers of the core show gradual degeneration to form the granular zone (*g.z.*). Two ascogenous hyphae (*a.h.*) are shown burrowing into the granular matrix of the zone. Other ascogenous hyphae are pushing in between the filaments. The nuclei in the ascogenous cells are seen most frequently in pairs.  $\times 1,400$ .

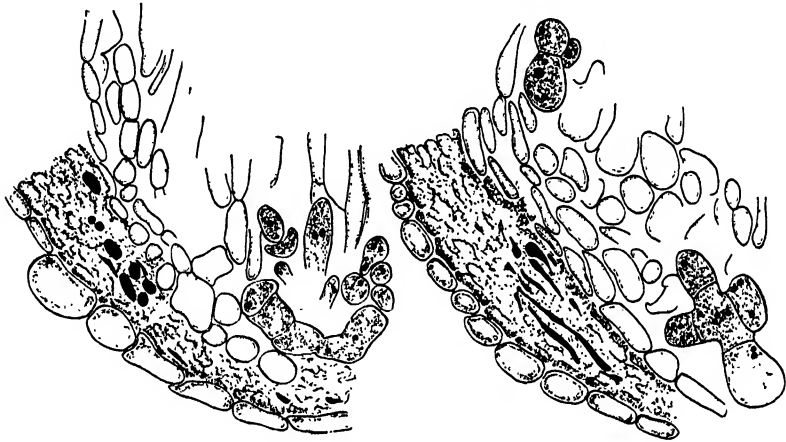
active growth. It is true that this parenchyma does eventually become distorted, but that change occurs at the time of active formation of ascogenous hyphae. Such cells, however, instead of appearing young and undeveloped, show all signs of maturity. Furthermore, the exact limit of each constituent cell is sharply defined by a definite constriction in the chain, which would not be the case if the supposed compound cell represented a hypha of irregular outline.

#### THE DEVELOPMENT OF ASCOGENOUS HYPHAE.

The mode of formation (as above described) of the tissues of the core, and their close aggregation within it, will no doubt ensure the juxtaposition of a number of cells which had their origin from different hyphae that intruded into the coil, and it is conceivable that conjugation takes place only between such cells of distant origin. If such be the case, it is evident that this renders possible the association also of distantly related nuclei within the conjugated cells. As previously stated these nuclei are seen to vary somewhat in size. It is frequently found that those of smaller size become associated in pairs, while in other cases examples are seen of small and large nuclei in comparative isolation (Text-figs. 3, 5, 6 ; Pl. XVIII, Fig. 9). After careful search it is impossible to state with certainty that nuclear fusion occurs within these conjugated cells. Casual observation would lead one to suspect that the larger nuclei are formed as the result of the fusion of the smaller ones, but though the latter are frequently seen in close contact, yet no reliable examples of their fusion have been detected. The association of nuclei in pairs is by far the more common occurrence here, yet the presence of so many isolated nuclei cannot be ignored. It is noticed that the latter are frequently somewhat bigger than the paired nuclei, and others again are strikingly large and very deeply stained. In the absence of reliable evidence of fusion it is extremely difficult to account for this disparity in size. It is possible that a progressive increase in the volume of some of the nuclei takes place as a consequence of failure to pair at cell-conjugation, and that such large nuclei are undergoing degeneration. Such abnormally large and deeply stained nuclei are described by Brooks (5) in *Gnomonia* and by Blackman and Welsford (3) in *Polystigma*. Brooks is of opinion that they are nuclei which have probably increased in size preparatory to division or that they are in a state of hypertrophy.

Further changes now take place in the appearance of the tissues at the core. The cells of the vacuolated parenchyma seem at first sight to be entirely devoid of contents, but they still contain a quantity of cytoplasm of extreme tenuity in which, however, the minute nuclei are very evident. Large intercellular spaces brought about probably by the growth and general expansion of the perithecium appear between them (Text-fig. 5). Closer

examination shows that the vacuolated cells are separating from each other only along the sides parallel to the long axis of the perithecium. These cells, previously isodiametric, very soon become elongated in the same direction, and in consequence appear as so many filaments or paraphyses of irregular growth with their direction towards the position of the future ostiole. It is possible that the paraphyses-like appearance of these filaments (which persist until the perithecium is mature) has led the systematists astray in the classification of *Ophiobolus*. These filamentous structures are really homologous with the conjugating cells. They are probably of the same



TEXT-FIG. 6. Portions from two neighbouring sections showing the complete conversion of the limiting layers of the core into granular matrix in which disintegrating portions of cell-wall material are represented by small wavy lines. Within the granular zone are seen remains of dark-stained ascogonial cells—the portions in the right-hand figure probably represent trichogynes. Ascogenous hyphae within the core.  $\times 1,400$ .

nature and origin as the 'interascicular parenchyma' mentioned by Stevens (31) in *Desmotascus*. The latter designation is hardly justifiable, for had the investigator traced the development of this parenchyma he would probably have found that it appeared at a stage considerably earlier than ascus formation.

With increased transparency in the core, due to mutual separation of the filaments, it is evident that rapid growth is taking place in the regions occupied by the conjugated cells. These are now seen to be invested with tangled chains of thick dense hyphae, the further development of which shows that they are the ascogenous hyphae emanating from the conjugated cells (Pl. XVIII, Fig. 9).

Concurrently with the formation of the ascogenous hyphae, and frequently somewhat earlier, the limiting zone of the core loses its cellular character and finally appears almost homogeneously granular. Such granulation commences in the innermost layer and is progressive until all the cells of the zone have become disintegrated. So striking is this appearance, and so definitely

localized, that it may now be referred to as the granular zone. Its presence in the Pyrenomycetes has been recognized by the earliest workers—van Tieghem (33), Zopf (35), and Oltmann (26) discovered it in *Chaetomium*, Fisch (14) in *Xylaria*, Bauke (1) in *Pleospora*, and later Dangeard (6) describes it in *Sordaria* and other types investigated by him. These writers have not, however, described the mode of formation of this zone, and Dangeard seems to have been the first to point out its probable correct function as a nutritive tapetal layer, but he describes it as first appearing at the moment of ripening of the asci. The conversion of the limiting layers of the core into the granular zone is initiated by dissolution of the cell-walls in these layers, and consequently the cell-protoplasts become isolated. The latter at this stage stain very faintly with the haematoxylin, whereas the spaces between them are occupied by small particles of cell-wall material staining a delicate pink with Congo red (Text-fig. 6). The cell-walls are evidently undergoing a process of digestion, and consequent upon this disintegration, which is more advanced in those parts of the zone which are in the immediate vicinity of the first-formed ascogenous hyphae, it becomes clear that these hyphae are now induced to continue their growth by burrowing into the granular matrix of the zone (Text-fig. 5). It is possible that the ascogenous hyphae are not only nourished in this way, but primarily, perhaps, this disintegration enables them to spread and ramify within the zone, so that an even distribution of hymenium is established. The hymenium is organized with greater facility towards the immediate base of the core, and it is here that the young asci first make their appearance (Pl. XVIII, Fig. 10).

#### DEVELOPMENT OF THE ASCI.

The nuclei in the ascogenous hyphae are generally arranged in pairs, but it is by no means rare to find isolated nuclei in them (Pl. XVIII, Figs. 9, 10). These hyphae are very irregular and tortuous in their course, and when they become septate their cells are generally binucleate, though on rare occasions a few cells may be seen to contain both paired and isolated nuclei (Text-figs. 5, 6; Pl. XVIII, Figs. 9, 10). The phenomenon of crossier formation is frequently seen (Pl. XVIII, Figs. 12, 13), but it does not appear to be exclusively adopted in the process of ascus formation. Even when it does take place the penultimate cell is not always delimited by cross-walls from the stalk and terminal cells (Pl. XVIII, Fig. 11). The two nuclei in the penultimate cell stand out prominently, and this is the first time in the investigation that nuclear structure has shown itself (Pl. XVIII, Fig. 12). These nuclei are furnished with a distinct reticulum and nucleolus within a clear cavity bounded by dense cytoplasm. The nuclei in close contact (Pl. XVIII, Fig. 13) are seen to merge into each other; this is followed by the union of the nucleoli, which appear to flow together, and by the intermingling of the chromatin. It is concluded that this is the only stage at which nuclear fusion in *Ophiobolus*

takes place. Evidence is not wanting that any binucleate cell in an ascogenous hypha is capable of forming an ascus directly without the intervention of a hook, as described by Maire (21), Faull (13), and Brooks (5). The figures (Pl. XVIII, Figs. 14 and 15) also show that the terminal cell of a hook may continue its growth as first described by Fraser (15) to form another ascogenous hypha.

#### CYTOLOGY OF THE ASCUS.

The cytoplasm in the young ascus is somewhat denser towards the apex, and it is here that the ascus nucleus takes up its position. It is remarkable that very few instances have been seen of the fusion nucleus in a state of rest, for as soon as the ascus is differentiated, the nucleus seems to enter almost immediately the state of 'first contraction'. The seriation of the nuclear phases seems to follow very faithfully the well-known procedure described by Farmer and Moore (11).

In the prophase of the first division (Pl. XIX, Fig. 16) the chromatin thread is fairly evenly distributed within the nuclear space. Its mode of entry into the first contraction phase (Pl. XIX, Fig. 17), by falling back in loops, is strongly suggestive of continuity. The synaptic stage is completed by a further contraction of the thread towards the nuclear membrane, but some parts of it may still stretch out into the cavity (Pl. XIX, Fig. 18). As the synaptic tangle loosens, a very characteristic feature is the presence in the delicate thread of a longitudinal split which, however, is not equally well seen in all its parts. At some points narrow elongated loops are evident, at others there is no sign of a split. Where the split is manifest, small chromatic granules seem to join contiguous filaments, but on account of their small size it is impossible to detect whether they also are divided (Pl. XIX, Fig. 19). An almost constant feature at this stage is a decided paleness of the nucleolus.

With the disappearance of the longitudinal split, the thread now enters the second contraction phase. It now appears considerably thicker, very deeply stained, and drawn into a tight mass near the nuclear periphery, usually on the side remote from the nucleolus (Pl. XIX, Fig. 20). The thickened thread frequently appears in the form of loops at the nuclear periphery (Pl. XIX, Fig. 21), and at this phase the whole nucleus has decreased considerably in volume. As the contraction passes off (Pl. XIX, Fig. 22), the longitudinal split is again faintly evident. Finally, the deeply stained thread segments itself transversely into four bivalent chromosomes which show the forms characteristic of the heterotype division (Pl. XIX, Figs. 23, 24).

#### THE FIRST DIVISION IN THE ASCUS.

The spindle at the first division in the ascus is very long and exceedingly narrow. It occupies a position parallel to the long axis of the ascus

and appears to be slightly curved in the form of an elongated S (Pl. XIX, Fig. 25). A small centrosome appears at each pole; this is frequently discoid in shape, at other times it appears like a small cap having its concave side towards the nuclear cavity. The chromosomes in the meantime have undergone considerable contraction, and their mode of arrangement at metaphase appears to be that usually presented by heterotype chromosomes at this stage. After careful count, there are four chromosomes at the metaphase (Pl. XIX, Fig. 25), and at the anaphase eight small chromosomes can be counted (Pl. XIX, Fig. 26). At telophase the chromosomes are densely massed together (Pl. XIX, Fig. 27). The ascus in its binucleate condition is very infrequently found, and as shown in Pl. XIX, Fig. 28, it is probable that the two nuclei enter the second division stage from late telophase without a period of rest.

#### THE SECOND DIVISION IN THE ASCUS.

The two spindles at this stage are slightly shorter than that at the first division (Pl. XIX, Fig. 29). At metaphase—the stage most commonly seen—the chromosomes are smaller than at the first division. At telophase (Pl. XIX, Fig. 30) the chromosomes are again densely massed together. The four nuclei resulting from this division become definitely reorganized, and show a distribution of chromatin in the nuclear cavity (Pl. XIX, Fig. 31).

#### THE THIRD DIVISION IN THE ASCUS.

The four spindles initiating the third division do not show any definite orientation within the ascus. The figures (Pl. XIX, Fig. 32) show the chromosomes at metaphase, and clear examples of anaphase were not seen. The eight nuclei formed at the end of this division are rather small but clearly defined (Pl. XIX, Fig. 33). They are oval in shape and show a nucleolus together with a small amount of chromatin within the nuclear space. They are fairly evenly distributed in the homogeneous cytoplasm of the ascus. From a consideration of the foregoing account of the division of the fusion nucleus in the ascus, it is concluded that meiosis and consequent reduction in the number of chromosomes has occurred during the first division. No stages of further reduction have been detected.

#### THE DEVELOPMENT OF ASCOSPORES.

Soon after the appearance of the eight nuclei in the ascus the cytoplasm is seen to show definite lines of cleavage. The segmentation (Pl. XIX, Fig. 34) seems to be initiated by the formation of long narrow vacuoles at certain points immediately within the ascus wall. At this stage it is seen that the small nuclei are seemingly reduced to mere nucleoli, and these are now closely pressed against the cell-wall. From a consideration

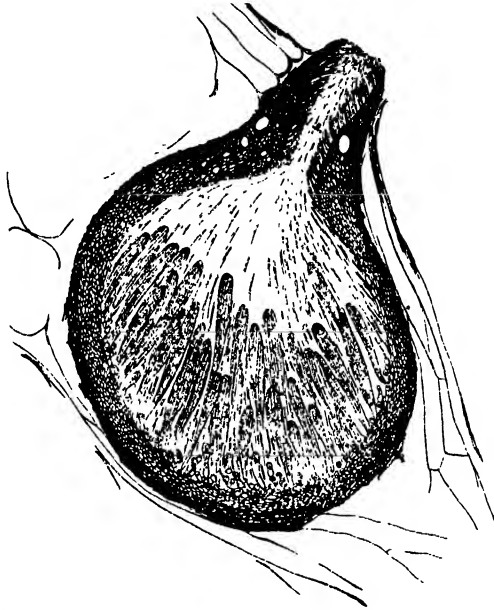
of Fig. 35, which is taken to represent a later stage, the cytoplasmic masses become rounded off by the expansion of these vacuoles. At one extremity of each of these ovoid masses, there now appears a centrosome with its astral rays, and from these observations it is probable that the process of cytoplasmic segmentation is initiated by vacuolation, and that it is possibly assisted by the later appearance of the astral rays. Finally, the delimitation of the spores does not seem to arise from the growth and fusion of the astral rays, and the whole process seems more in agreement with the views of Faull, than with those of Harper, on spore formation in the Ascomycetes.

Before the cytoplasmic masses proceed to elongate towards the apex of the club-shaped ascus, there is still present at its base a small amount of frothy protoplasm (Pl. XIX, Fig. 35). This soon disappears, and when the spores are in the form of a fascicle, a transverse section of an ascus (Pl. XIX, Fig. 39) shows that they are separated by a quantity of epiplasm which has probably been distributed from the frothy mass at the base. The fascicle of ascospores exhibits a distinct torsion within the ascus, and the single nucleus in each spore takes up a position at its centre. The nucleus consists of a fairly large, deeply stained spherical body, in association with which is a small amount of dark chromatin of indefinite form (Pl. XIX, Figs. 36, 37). It soon proceeds to divide, and favourable preparations show that division takes place in a mitotic manner. The spindles are exceedingly small, and the metaphase stage shows the presence of four minute chromosomes (Pl. XIX, Fig. 38). At maturity, the ascospores (measuring about 70 to 80  $\mu$  by 3 to 5  $\mu$ ) are septated usually into six cells of fairly uniform length, in each of which can be seen with great clearness a single nucleus (Pl. XIX, Fig. 40). Nuclear division in the spore was seen only at the primary stage described above, and there is no doubt that subsequent divisions are also mitotic. During the process of septation and at maturity, the spores are exceedingly rich in food reserve, principally in the form of droplets of oil.

#### SPORE DISCHARGE.

In preparation for the discharge of ascospores certain changes have taken place in the tissues which are situated within the neck of the perithecium. In this region, the outer layers of the perithecial walls are closer together and are seen to overarch those immediately below them, which, however, are not so closely compacted. In other words, the inner layers of the neck are in the form of an elongated cone covered over at the apex and flanks by the more compacted outer cells. The cells within the cone now split apart and separate right down to the apex of the central core, after which they become considerably elongated and attenuated. With general

expansion of the perithecium, this cone of tissue becomes hollow in order to form the neck-canal, and into the latter the attenuated cells project in an upwardly oblique direction to form the periphyses. Concurrently with their formation, the filamentous parenchyma at the apex of the core has become greatly distorted and shrivelled, so that a hollow space appears above the hymenium. Consequently, expansion at the base of the neck will now put the neck-canal in communication with the core. During the formation of the periphyses, it is found that growth at the seat of the future ostiole is not



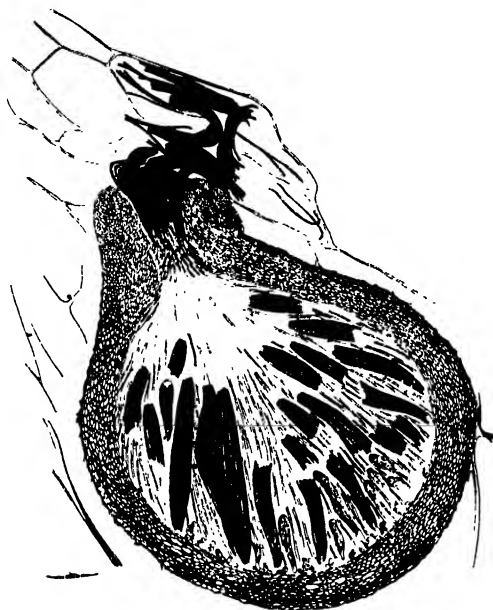
'TEXT-FIG. 7. Median longitudinal section of a perithecium (within the leaf-sheath) at time of formation of spores; the interior is lined with hymenium and ascogenous cells; the filaments radiate towards the ostiole, into which project the periphyses; the neck has broken through the overlying host tissues. (From a micro-photograph.)

equally centrifugal. This inequality of growth causes the neck of the perithecium to become slightly curved, an adaptation which no doubt enables the neck to break through the overlying host tissues. While the perithecium is still closed, the formation of ascospores is initiated. Later, when a considerable number of spores are mature, a few cells still closing the ostiole break down, and so enable spore discharge to take place (Text-fig. 7).

At maturity, the interior of the perithecium is lined with hymenium and the latter contains asci at all stages of development from the short ascogenous hyphae at the base (Text-fig. 7). A striking feature in the ascus wall is the presence, at the apex, of a small thick ring (Pl. XIX, Figs. 34, 35, 36) which takes a bright red colour with the Congo stain. It makes its



appearance in the wall at a very early stage, frequently when the ascus is seen to contain the spindle of the first nuclear division. The small ring appears at a zone situated slightly behind the apex of the ascus, and the small dome-shaped part of the wall at the tip remains very evident. The ascus wall shows a slight constriction at the insertion of the ring, but I have not been able to establish that the dome-shaped cap ever becomes perforated to form an apical pore. When the ascospores are ripe, a single spore is much wider than the whole diameter of the ring, and I have not seen the



TEXT-FIG. 8. Median longitudinal section of a mature perithecium at the time of spore-discharge. The eight-spored bundles are extruded *en masse*. They remain at the ostiole for some time before liberation into the soil. (From a micro-photograph.)

latter in a state of expansion that would permit the escape of at least a single spore. It is very significant that one never finds empty asci in the perithecia of *Ophiobolus*, a phenomenon observed also by Zopf (35) in *Chaetomium*. The sequence of events in *Ophiobolus* seems to be initiated by the complete dissolution of the ascus wall, but despite this gelatinization the apical ring still remains intact at the top. Its persistence at the apex, and the manner in which the tips of the spores cling closely to it, suggested that a portion of the ascus wall still remained in the form of a thin cap over the spores, but this could not be clearly established. It is, however, exceedingly difficult to get the wall to stain after such gelatinization has set in, and one can only see a hazy effect of the stain between the ripe asci. This appearance is probably due to the accumulation of a matrix derived

from the disintegrating walls of the ripe asci. It is conceivable that by the absorption of moisture through the wall of the perithecium (the basal part alone is enclosed within the host), the first effect of such absorption from without would be to cause the gelatinous matrix to swell immediately around the *bases* of the spore-bundles. Under the directive influence of the flask-like contour of the perithecium interior, together with the radiate arrangement of the filaments and periphyses towards the ostiole, the preparations show very clearly that the spore-bundles are extruded *en masse*. Moreover, the marked torsion (Pl. XIX, Fig. 36) in the spore-bundles no doubt facilitates the extrusion of the spore-masses. It is a very frequent occurrence to find the small ring perfectly intact at the apex of an extruding spore-bundle, and it may probably have a function of securing rigidity at the tip of a bundle in a manner comparable to that of the cap at the apex of a root. This is the appearance presented in the neck at spore discharge (Text-fig. 8), and the spores remain at the ostiole for some time before dispersing. The manner of ascospore discharge from the perithecium has been studied by Mangin (24), Hori (17), M<sup>e</sup>Alpine (22), Waters (34), and Kirby (19). With the exception of Mangin, all these investigators agree that the spores are extruded at the ostiole, but that they are not shot forth as stated by him. Kirby has several times observed a dry mass of spores to have formed at the ostiole, where it remained for some months if there was no rain to dissolve it. Field observations, he adds, seem to indicate that the spores are discharged during rainy periods, and that usually the splashing rain removes them before they can accumulate at the ostiole.

#### GENERAL CONSIDERATIONS.

The investigation has shown that the ascogonial coils of *Ophiobolus*, consisting of a number of cells furnished with trichogynes, are abortive structures. The presence of a trichogyne naturally suggests association with fertilizing agents of the nature of spermatia produced in spermogonia. Pyrenomycete archicarps furnished with a trichogyne occur in *Gnomonia* (5), *Polystigma* (3), *Sphaerella* (16), in all of which spermogonia are developed, and it is not surprising, therefore, to find these organs present also in *Ophiobolus*. The minute size of the spermatia, their prominent nuclei, and the seeming absence of food reserve all appear consistent with the structure of male cells. In *Ophiobolus*, where they are seen in enormous numbers at the time of appearance of ascogonial coils, they do not seem to be functional. They are, however, not precluded from association with the ascogonia, for at that time the investment of the latter with vegetative hyphae has not begun. In the present investigation such association has not been observed, and it is highly probable that the primitive mode of fertilization by means of spermatia has been replaced by a reduced process, the essential feature of which is the approximation of vegetative nuclei. This view is strongly

supported by the classical works of Blackman on the Uredineae and of Farmer, Moore, and Digby on apogamy in the Ferns.

From a review of the Pyrenomycetes, we find that Fisch (14) in *Xylaria* recognized a primordial hyphal coil, called by him a 'Woronin-hypha', which became disorganized, but he was unable to establish any genetic connexion between this structure and the ascogenous cells. *Hyphoxylon*, closely allied to *Xylaria*, has, however, been described by Lupo (20) as having an ascogonium which develops from the cells of a 'Woronin-hypha'. He states that these cells round off, increase in size, and eventually separate from each other to form the ascogonia from which arise by budding the ascogenous hyphae. There are points of close similarity between Lupo's account and the present observations, with the radical difference, however, that the large round cells (the conjugating cells) in *Ophiobolus* are traceable at their first appearance to vegetative hyphae, and not to the ascogonial coil, which may here be compared to Lupo's 'Woronin-hypha'. Bauke (1) stated, in the case of *Pleospora*, that the perithecium was formed by differentiation, at a late period of growth, of a spherical mass of tissue originally composed of a uniform parenchyma. His conclusions are in the main identical with those arrived at here, but he does not appear to have traced the derivation of this parenchyma. In *Claviceps* again, Fisch (14) states that development takes place without ascogonia. From the present observations it appears that *Ophiobolus* is another type which may be included with an increasing number of Ascomycetes in which normal sexuality has disappeared. In the group Pyrenomycetes, it is in this respect very closely allied to *Gnomonia* and *Polystigma*. Unlike the former type, but in agreement with the latter, the ascogonial coils persist for a long period during perithecial development. In their discussion of these types Brooks (5) and Blackman and Welsford (3) state that the ascogenous hyphae may quite possibly arise by differentiation of ordinary cells of the vegetative mycelium. The present investigation may possibly have established this phenomenon in *Ophiobolus*, where the derivation of ascogenous hyphae has been observed to take place from vegetative cells in conjugation.

The observations set forth in this paper appear to be in entire agreement with Saccardo's diagnosis of the species *O. graminis* (30). Engler and Prantl (10), however, classify the genus within the Pleosporaceae, but as the present investigation has shown that there are no true paraphyses, their table modified accordingly would be: (a) Perithecia without a stroma and sunk in the substratum; (b) Asci usually thickened apically, opening by a pore (*sic*), perithecia usually beaked; (c) Perithecia without a clypeus. Gnomoniaceae. From most of the considerations presented in this paper, the genus should be regarded as belonging to this family, and, moreover, the investigation has revealed cases of striking parallelism with the results obtained by Brooks in his work on *Gnomonia*.

## SUMMARY.

1. *Ophiobolus graminis* causes a disease on oats. The mycelium is intracellular and consists of uninucleate cells.

2. The mycelium is capable of penetrating the host cells, but no haustoria are developed.

3. Spermogonia and spermatia are formed. They are considered to be functionless structures.

4. The mycelium forms small coils which give rise to a number of archicarps, each furnished with a trichogyne. Subsequent development shows that the archicarps are abortive.

5. Perithecial development takes its origin from vegetative hyphae which intrude into the space bounded by an ascogonial coil. The latter remains for a considerable time during development.

6. The perithecia appear on the host singly, or in groups of two or three. They are ovoid in shape, and furnished with a long, smooth, and curved beak. The external walls are carbonaceous.

7. The phenomenon of reduced fertilization or apogamy takes place. It is believed to be initiated by the conjugation of two or more vegetative cells. From such conjugated cells the ascogenous hyphae arise.

8. There is no fusion of nuclei antecedent to ascus formation. The nuclei of the ascogenous hyphae appear most frequently in pairs.

9. Ascus development appears to take place from any binucleate cell of an ascogenous hypha.

10. The only nuclear fusion that has been observed occurs in the young ascus.

11. The cytology of the ascus is described. Meiosis and reduction of chromosomes is effected at one stage only, viz. the first nuclear division. It is believed that four chromosomes appear at all the stages of division.

12. The ascospores are septated usually into six uninucleate cells; they are hyaline and guttulate.

13. The phenomenon of spore-discharge is described.

14. It is suggested that the genus *Ophiobolus* be transferred from the Pleosporaceae to the Gnomoniaceae.

The writer wishes to express his deep obligation to Mr. D. W. Davies, B.Sc., Advisory Mycologist, for supplying the material for this work; to Professor T. H. Parry-Williams, M.A., B.Litt., Ph.D., and the Rev. E. E. Thomas, M.A., D.Litt., for their kind help with the literature; and to his colleague Mr. P. W. Carter, M.Sc., for much assistance in preparing the material for investigation. He is also greatly indebted to Professor Lloyd Williams for advice and criticism during the progress of this research.

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## EXPLANATION OF PLATES XVIII AND XIX.

Illustrating Dr. S. G. Jones's paper on the Development of the Perithecium of *Ophiobolus graminis*, Sacc.

All figures have been drawn with the aid of the camera lucida, with a Koristka 2 mm. objective, N.A. 1. 3, and comp. oculars 6, 8, 18.

### PLATE XVIII.

Fig. 1. Spermatia.  $\times 3,500$ .

Figs. 2, 3. An ascogonial coil from two neighbouring sections showing a number of archicarps at the centre and surrounded by portions of vegetative hyphae.  $\times 3,500$ .

Figs. 4, 5. A single ascogonial coil from two neighbouring sections; the break in the sections is indicated by the asterisks. The coil has formed two archicarps towards the space bounded by it. In Fig. 5 the large cell is an oogonium, and the beak a trichogyne. One nucleus in this cell is probably about to pass into the beak, the other to remain in the oogonium. Uni- and binucleate vegetative hyphae are seen to intrude into the space bounded by the coil. Note differentiation in the density of the cytoplasm.  $\times 3,500$ .

Figs. 6, 7. Neighbouring sections of a young perithecium. Fig. 6 is median, and Fig. 7 tangential. In the former is shown at the centre the aggregation of vegetative cells to form the 'core', and the dark ascogonial coil is pushed outwards. The whole is surrounded by loosely arranged hyphae which form the wall of the perithecium.  $\times 2,800$ .

Fig. 8. Portion of the core of a young perithecium showing two of its cells in complete conjugation, and abutting on them is the vacuolated parenchyma in the core.

Fig. 9. The basal part of the core tissues showing ascogenous hyphae arising from multinucleate conjugated cells. The latter are indicated by the two asterisks, and the cytoplasm in the ascogenous hyphae is much denser than that in the conjugated cells. The tips of two ascogenous hyphae are shown burrowing into the granular zone, the wavy lines in which indicate disintegrated cell-wall material. Note the paraphysis-like vacuolated parenchyma between the dense hyphae.  $\times 2,800$ .

Fig. 10. Young asci with the nuclei in the 'first contraction' stage.  $\times 2,800$ .

Fig. 11. Showing a four-nucleate terminal cell of an ascogenous hypha, with a 'bulb' at the position of the penultimate cell to form probably an ascus which will receive the paired nuclei below. There is no delimitation of stalk and terminal cells.  $\times 3,500$ .

Fig. 12. Crosier-formation, showing the binucleate penultimate cell, and the terminal cell as yet not cut off by a cross-wall from it. The two nuclei show well-marked differentiation into nucleoli and chromatin.  $\times 3,500$ .

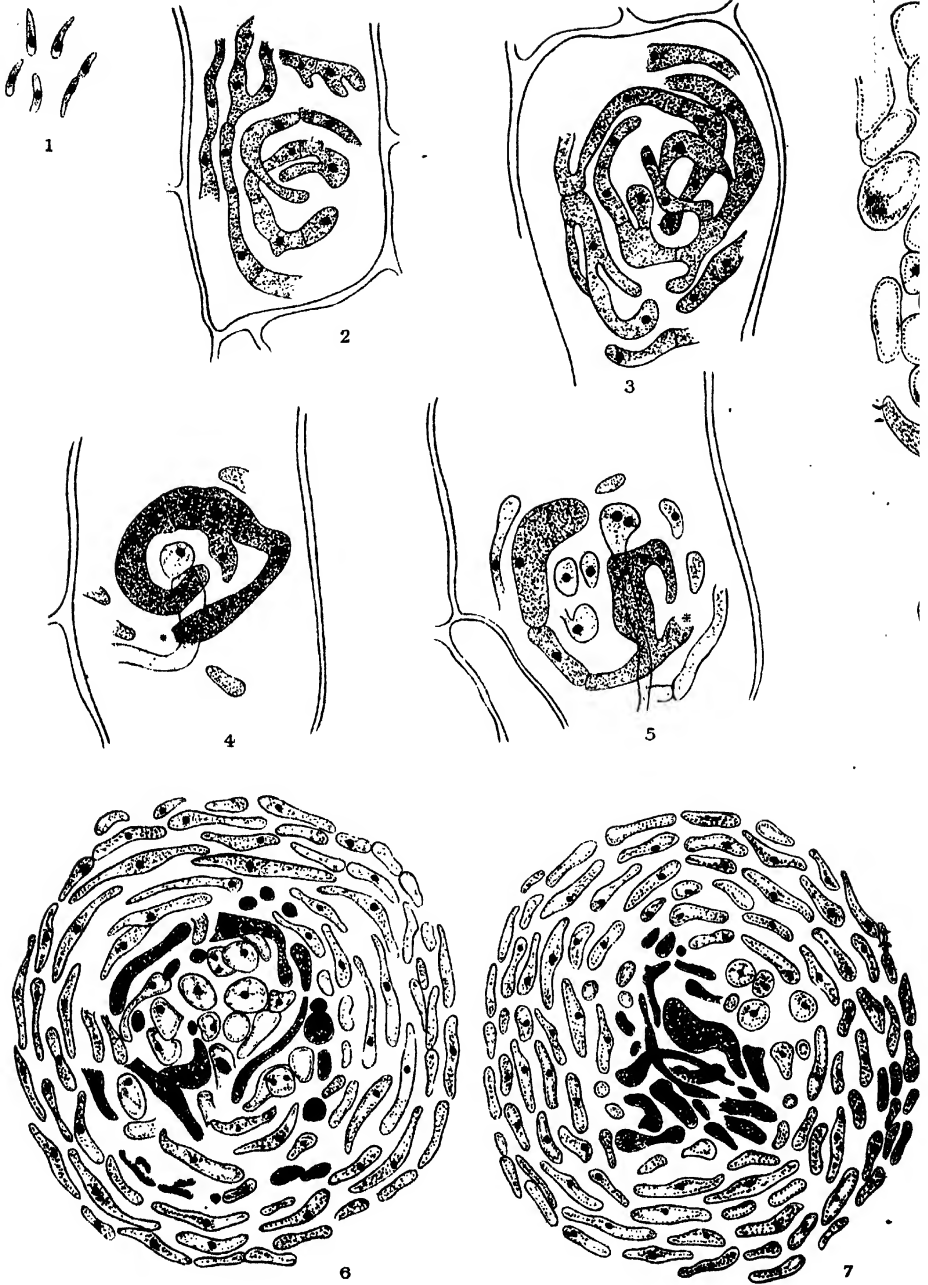
Fig. 13. A normal crosier showing the paired nuclei in the penultimate cell about to fuse.  $\times 3,500$ .

Fig. 14. A young ascus subtended by an elongated terminal cell which is probably growing out to form an ascogenous hypha.  $\times 3,500$ .

Fig. 15. A young ascus with two nuclei in a 'bulb' at its base.  $\times 3,500$ .

## PLATE XIX.

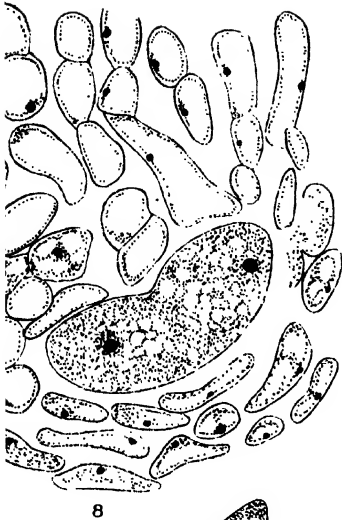
- Fig. 16. Prophase of first nuclear division in the ascus.  $\times 3,500$ .  
Fig. 17. First contraction.  $\times 3,500$ .  
Fig. 18. Synapsis.  $\times 3,500$ .  
Fig. 19. Stage showing the longitudinal split in the spireme with chromatic beads ; note paleness of the nucleolus.  $\times 3,500$ .  
Fig. 20. Second contraction.  $\times 3,500$ .  
Fig. 21. Second contraction and formation of loops.  $\times 3,500$ .  
Fig. 22. Chromatin emerging from second contraction, showing at some points reappearance of the split.  $\times 3,500$ .  
Figs. 23, 24. Four bivalent chromosomes showing characteristic forms of heterotype division.  $\times 3,500$ .  
Fig. 25. Spindle of the first division showing 'metaphase'.  $\times 3,500$ .  
Fig. 26. 'Anaphase' showing eight chromosomes on the spindle.  $\times 3,500$ .  
Fig. 27. 'Telophase'.  $\times 3,500$ .  
Fig. 28. The two nuclei in process of reorganization from telophase.  $\times 3,500$ .  
Fig. 29. The two spindles of the second division, showing 'metaphase' in the top spindle and probably 'anaphase' in the lower.  $\times 3,500$ .  
Fig. 30. 'Telophase' of second division.  $\times 3,500$ .  
Fig. 31. Four resting nuclei terminating the second division.  $\times 3,500$ .  
Fig. 32. Four spindles of the third division. The stages are those of metaphase.  
Fig. 33. Eight nuclei in the ascus ; thickened ring at apex.  $\times 2,800$ .  
Fig. 34. Migration of the nuclei to the ascus wall and cleavage of the cytoplasm ; appearance of elongated vacuoles.  $\times 2,800$ .  
Fig. 35. Appearance of centrosomes and astral rays at the terminations of the spore-masses, accompanied by increase in volume of the vacuoles.  $\times 2,800$ .  
Fig. 36. The fascicle of uninucleate ascospores showing torsion.  $\times 2,800$ .  
Fig. 37. Portion of a spore-bundle showing nucleoli and elongating chromatin.  $\times 2,800$ .  
Fig. 38. Division of the primary nuclei in the ascospores ; one spindle shows four minute chromosomes at the 'metaphase'.  $\times 2,800$ .  
Fig. 39. Transverse section of young ascus showing epiplasm around the spores.  $\times 3,500$ .  
Fig. 40. Ripe ascospore septated into six uninucleate cells and containing oil globules.  $\times 2,800$ .



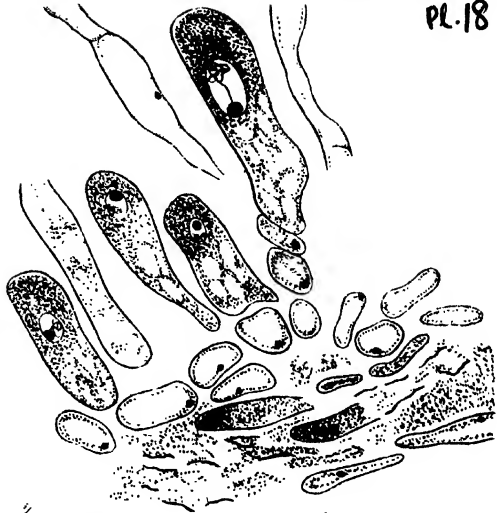
S.G.J.

JONES—OPHIOBOLUS.

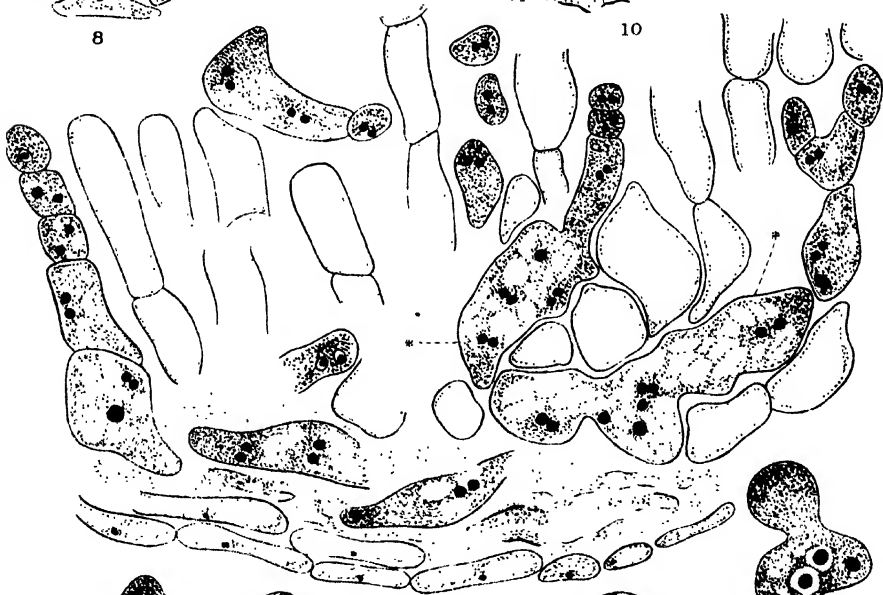




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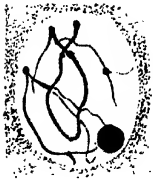
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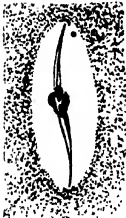
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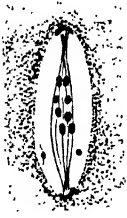
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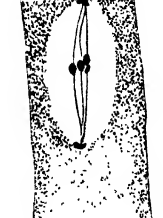
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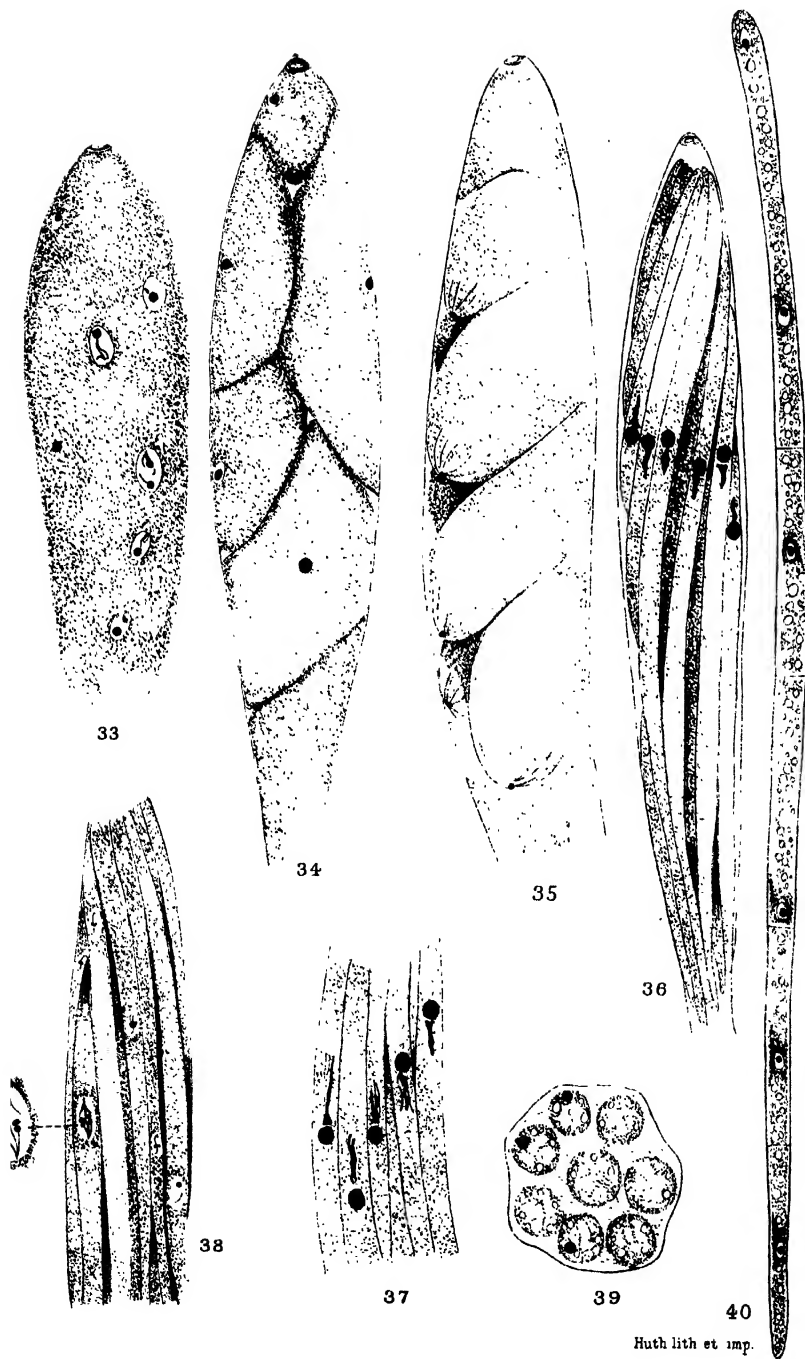


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S.G.J. del





# Excretory Systems in the Secondary Xylem of Meliaceae.

BY

PERCY GROOM, D.Sc., F.R.S.

With Plate XX and five Figures in the Text.

## A. HISTORICAL.

THE first detailed description of the excretory tissue dealt with in this paper appears to have been given by Moeller (1876), who found in the secondary xylem of the stem of *Carapa guianensis* certain excretory reservoirs that he described as 'resin-sacs' (Harzlücken). According to him the excretion results from the metamorphosis of the walls of wood-parenchyma, and arises first as a honey-yellow intercellular substance, and subsequently increases in quantity owing to the participation of larger masses of cells and dissolution of their walls, until finally the wall of each cell is completely dissolved and the cell is represented by the excretion enveloping a granular dark-brown substance that is the remnant of the original cell-contents. The gland or reservoir is therefore, according to Moeller, lysigenous in origin and the excretion is resinous.

H. H. Janssonius describes the 'resin-passages' (Harzgänge) in the secondary wood of the stem of three meliaceous genera and species: *Carapa obovata*, Blume (*C. moluccensis* of 'Index Kewensis'), *Melia Bogoriensis*, Koord et Valet., and *Sandoricum indicum*, Cav. The following is the description supplied by Janssonius of these resin-passages. They form tangential sheets, and in transverse section each almost fills the space between two adjoining medullary rays, but in *Sandoricum* sometimes the space is not nearly filled by the small resin-passages. In *Carapa* and *Melia* the tangential faces of the passage are bounded by wood-parenchyma. The excretion is light yellow and homogeneous, and in *Carapa* and *Sandoricum* that portion of it adjacent to the surrounding parenchyma stains red with phloroglucin and hydrochloric acid. In *Carapa* and *Sandoricum* the resin-passage is schizogenous in origin, but the subsequent enlargement

is lysigenous: in these two species more than one passage may occur at first between two adjoining medullary rays, but they subsequently fuse, thanks to their lysigenous enlargement. In these two species parenchyma cells are often embedded in the excretion. The cells of the medullary rays close to the secretion are not dissolved in *Melia*, but are occasionally so in the other two species. The contents of the wood-parenchyma and medullary rays immediately adjoining the resin-passages agree with those in other parts of the xylem in the case of *Carapa* and *Sandoricum*, but include an additional red-brown substance in *Melia*.

Janssonius also described certain 'secretory sacs' (Sekreträume) in the secondary wood of the stem of *Cedrela febrifuga*, Blume, var. *glabrior*. According to him the growth-ring of this variety is clearly recognizable by, *inter alia*, the largest vessels being ranged as a zone (circumferential) at the inmost part of the growth-ring. He states that the secretory sacs occur solely in places that are elsewhere occupied by these largest vessels, and definitely adds that they take their origin in these latter, but subsequently widen by dissolution of the surrounding parenchyma cells. The secretion is light yellow, homogeneous, and stains red with phloroglucin and hydrochloric acid (Janssonius makes no distinction between the staining reaction of the marginal and centre parts of the secretion). Some of the parenchyma cells bounding the excretion project into the sacs in a manner recalling tyloses, and their bladder-like projecting parts have thick lignified walls that bear numerous simple pits. Cells of the medullary rays never undergo dissolution. The wood-parenchyma immediately adjoining the sacs contains starch, small quantities of a red-brown substance, and tannin. Janssonius describes the structure of these sacs as being precisely like that of *Carapa*. Of the sacs of *Cedrela febrifuga*, var. *velutina*, he merely states that the secretion is brown and that the medullary rays in them undergo dissolution.

Moeller and Janssonius (except in the cases of two varieties of *Cedrela*) agree in applying the term 'resin' to the excretion, but differ as regards the precise mode of origin and development of the 'resin-passages'.

Excretory sacs or veins are familiar to timber-merchants in this country as occurring in American and West African mahoganies belonging respectively to the meliaceous genera *Swietenia* and *Khaya*, and are commercially known as 'gum-veins' or 'gum-streaks'. In the heart-wood of the West African meliaceous *Lovoa Klaineana* (so-called West African or Benin walnut) they are so regularly present and obvious as black lines on the transverse and radial sections that they serve as a means of identification of the timber and depreciate its commercial value.

The structure of the veins in *Swietenia*, *Khaya*, and *Lovoa* has not been previously investigated.

I found that the excretory sacs similar to those described by Moeller

as present in the wood of the trunk of *Carapa guianensis* occur in the erect pneumatophores, which are probably outgrowths of a root, of *Carapa moluccensis*, var. *gangetica* (Groom and Wilson).

## B. PRESENT INVESTIGATION.

The present investigation was confined to an examination of the structure of the secondary xylem of the trunk of *Lovoa Klaineana* and of the erect pneumatophores of *Carapa moluccensis*, var. *gangetica*. Throughout the text in the sequel the term wood therefore denotes secondary xylem.

### LOVOA KLAINEANA.<sup>1</sup>

The wood of this species shows a clear distinction into walnut-brown heart-wood and lighter-coloured sap-wood. It has a double-spiral grain.

The excretory tissue under consideration is visible to the naked eye in the heart-wood in the form of thin black sheets, revealed: in cross-section, as thin lines concentrically arranged round the organic centre, as complete circles (according to the testimony of timber-merchants) or shorter or longer arcs; in radial section, as longitudinal lines which run for considerable distances (according to the evidence of timber-merchants a single line running from one end of a long log to the other); in tangential section, as black surfaces. Hence the excretory tissue as seen by the naked eye appears to occur in the form of concentric complete cylinders or fractions of such. Under the microscope, however, each sheet of excretory tissue is seen to be perforated by medullary rays with or without wood-parenchyma round these, so that it is a fenestrated structure. Similar sheets of excretory tissue occur in the sap-wood, but are not obvious as they largely agree in colour with the general sap-wood.

In the specimen examined no growth-rings were detected, but I made no measurements to test their presence or absence.

In the same specimen the radial distances apart of the successive concentric rings of excretory tissue as seen in transverse section were, beginning at the part nearest the centre of the trunk, 1, 10, 3.5, 9, 16, 20, 7, 6.5 mm. In one case, however, the black line as seen by the naked eye was found under the microscope to consist of three concentric series of excretory glands. It thus appears probable that the production of these glands is no regular periodic phenomenon.

<sup>1</sup> This wood is easily procurable in England from timber-merchants, who sell it under the name of 'Benin' (or 'West African') walnut. The black lines marking the excretory sheets render identification easy. All the microscopic sections illustrated here were taken from seasoned timber after immersion in cellulose acetate dissolved in acetone, without any previous 'fixation'. Thus the commercial timber provides excellent laboratory material.

The wood consists of :

1. *Tracheae*: numerous, generally diffused, but either solitary or ranged in small, usually radial groups of 2-15 vessels.
2. *Wood-fibres*: non-septate, with lumina of moderate size, and with walls of moderate thickness and bearing simple pits.
3. *Parenchyma*:
  - (a) Ordinary wood-parenchyma: (1) metatracheal (concentric) in sheets 2-8 cells in radial thickness, but interrupted occasionally by isolated vessels; (2) paratracheal and sometimes completely surrounding a vessel (in transverse section); (3) scattered.
  - (b) Parenchyma containing inorganic crystals, the cells being small cubical and arranged in longitudinal rows.
  - (c) Parenchyma bordering on the excretion.
4. *Medullary rays*: fusiform in tangential section and 2-5 seriate (mostly 3-4 seriate), and containing in the heart-wood abundant tannin.

In the specimen examined the wood-vessels were much more numerous in the sap-wood than in the heart-wood; and in the former the metatracheal parenchyma was accordingly reduced in transverse section to short, isolated, tangential bands, and the concentric continuous line of glands was sometimes locally replaced by a short, somewhat irregular series of glands, not quite parallel to the contour of the stem, interrupted here and there by vessels.

Many vessels of the heart-wood contained a *plugging substance*, which was brown or brownish yellow in colour, and occurred in masses that either locally did or did not occlude the lumen: these masses often appeared to be more frequent in the vessel opposite to a medullary ray, as if the ray-cells were largely responsible for their production.

The wood-fibres and parenchyma show a strong tendency to be arranged, in transverse section, in radial rows: when parenchyma radially gives way to fibres the single radial row of parenchyma is apt to be replaced by two radial rows of fibres.

#### *Excretory System.*

##### *(a) Well-developed Excretory Tissue.*

Where the excretory tissue is well developed and occurs in the form of a fenestrated cylinder or part of such a cylinder, it resembles in form the resinous tissue of the secondary xylem of Conifers suffering from resin-bleet, for in this case a network of resin-sacs largely occupies the cylinder of wound-parenchyma which is perforated by medullary rays.

For the purpose of brevity of description the net-like tissue of *Lovoa*



will be described as if it consisted of a number of glands or sacs composing the meshes of the network.

In *transverse section* (Pl. XX, Figs. 1 and 2) each gland is irregularly rounded in outline, being frequently oval with the long axis tangential, and nearly fills the space between two adjoining medullary rays. In the centre is a yellowish mass of excretion which here and there may be interrupted by cells rounded in outline. On the radially outer and often on the inner sides of the excretion are radial rows of radially compressed parenchyma cells, which are more abundant on the outer side; these have straight walls, except where they abut on the excretion into which their walls bulge. Nearer the medullary rays the radial arrangement becomes obscured, as the orientation of the cells conforms rather with the shape of the excretion and their greater length is tangential to the outline of the latter, and their compression is in a direction perpendicular to this, so that when present and in actual contact with the rays, they are elongated parallel to the rays. In some cases the excretion abuts directly on the ray. Where the parenchyma is arranged in distinct radial rows, a row, especially near the excretion, often divides into two. It is often evident that the radial lines on the inner and outer sides of the excretion correspond, and originally were derived from the same cambium cell. The excretion is interrupted at places by isolated or often short tangential rows of cells (two to three in number), either projecting in from the margins or lying free in the interior: in some cases where the gland is small a tangential band of cells divides the excretion into two parts.

In *longitudinal radial section* (Pl. XX, Figs. 3-5) the persisting cells embedded in the excretion are seen to be ranged in longitudinal rows, which are either one or two cells in thickness. The rows run from one margin towards the centre, and then bend and either rejoin the same margin, or run across to the opposite margin, and may meet lines of cells coming from this, and yet continue their course. Here and there they gradually lose their distinctness and appear wholly or partially filled with excretion. Particularly in these last regions their walls have numerous abnormally large simple pits, which are much larger than those of most of the parenchyma cells, and are sometimes greatly elongated in surface view; these enlarged pits seem to represent incipient dissolution of the cell-wall that began at the pits. Where the gland abuts above or below on a medullary ray, the parenchyma cells bounding its terminal portion change in direction and arch over the actual tip, so that they here become parallel with the cells of the medullary ray, from whose cells they are distinguished by their much poorer contents. When the rays are closely superposed the intervening gland shows a central rounded mass of excretion surrounded by parenchyma, more or less curved, parallel to the periphery of the excretion (Pl. XX, Fig. 5).

*Structure of the excretion.* In all sections the excretion is marked according to a definite pattern. Radial and transverse sections (Pl. XX, Figs. 2-4) of the excretion show continuous longitudinal and radial lines of cell-shapes, as the excretion is composed of radial sheets of metamorphosed wood-parenchyma, each cell being represented by a brick-shaped mass (occasionally with a pointed end), which is hyaline except in the inmost region, where it is generally granular. The lysigenous mode of origin of the main mass of the excretion is also demonstrated by the fact that in longitudinal radial section, here and there a longitudinal row of persistent parenchyma cells is directly continuous with a similar row of brick-shaped excretory units. As pointed out by Moeller in *Carapa*, some of the parenchyma embedded in the excretion represents transitional stages between cell-structure and excretion. In some cases the excretion belonging to one cell assumes the form of a peripheral, more or less thick coat, invested or not by a persistent wall,<sup>1</sup> and surrounding a central cavity in which crystals or other remnants of cell-contents are lodged.

It is evident that plastic material, at the expense of which the excretion is partly produced, may reach the gland by way of wood-parenchyma or medullary rays, and be distributed in the interior of the developing excretion by the rows of parenchyma persisting within this.

At its margins the gland is schizogenous, and the excretion is present in the larger or smaller intercellular spaces between the parenchyma cells.

The medullary rays, each usually with a surrounding coat of wood-parenchyma, for the most part pass through the excretory sheet intact.<sup>2</sup> But in transverse sections sometimes there is presented a picture that seems to indicate that the ray-cells occasionally undergo metamorphosis into excretion; this appearance is delusive in some if not in all cases, as the section may really show partly disintegrated wood-parenchyma that is running parallel to, and in contact with, the ray-cells forming the upper and lower margins of the ray.

#### (b) *Feebly developed Excretory Tissue.*

Even when an excretory band as seen in cross-section is well developed, the fraction of the space lying between two neighbouring medullary rays that is occupied by the gland varies. In transverse section the gland may: (a) extend tangentially from ray to ray; (b) nearly extend tangentially from ray to ray, but be separated from these by a thin layer of parenchyma (Pl. XX, Fig. 8); or (c) occupy a smaller part of the space between the rays (Pl. XX, Figs. 6, 7), and may be replaced by two little glands, as is often the case when one or more wood-vessels invade the excretory band.

<sup>1</sup> It is often difficult to determine whether or not the cell-wall is present, as the excretion stains like the wall.

<sup>2</sup> Such is also the case frequently in coniferous wood that has undergone resinosis, and in cherry wood changed by gummosis.

In some cases in transverse section (Pl. XX, Figs. 6 and 7) the excretory band is so feebly developed as to escape notice until the microscope is used. Such feeble bands shed additional light upon the genesis of the excretory tissue.

In the first place it becomes clear that the excretory band corresponds to a band of metatracheal parenchyma whose cells are arranged in radial rows in transverse section. In one specimen a metatracheal band of parenchyma showed between each successive pair of rays, travelling circumferentially the following structural constituents ( $p$  = parenchyma,  $v$  = wood-vessel,  $g$  = gland):  $p$ ;  $p, lv$ ;  $p, g$ ;  $p, 3v$ ;  $p, v$  (a radial row);  $p$ ;  $p, g, v, p, g$ ), followed by twenty-six medullary rays between which there were no glands, but merely parenchyma interrupted here and there by single wood-vessels. In this particular metatracheal band the glands were very small, and in the spaces between two successive rays there was about the same number of tangential layers of parenchyma cells in glandless and gland-including spaces. Here it may be noted that even when an excretory band is well developed, its radial thickness does not exceed that of a glandless metatracheal band of parenchyma, although its circumferential extension is greater. From these facts it is evident that the stimulus evoking the production of excretory tissue does not cause a thickening of the metatracheal band, but does cause this to be lengthened circumferentially, and does cause more numerous radial and tangential divisions to take place in the cells actually, subsequently *either* undergoing metamorphosis into excretion, or constituting the parenchyma immediately surrounding this.

Simple glands as seen in transverse section in metatracheal bands showed the structural features detailed below. In interpreting these features it must, however, be remembered that the transverse section may represent either the tapering top or bottom of a gland which, in its middle part, may be more elaborate and larger, *or* the typical structure of a small isolated gland: yet in either case the picture presented is that of tissue that locally is only feebly glandular.

The smallest trace of a gland that I saw showed in transverse section three radial rows of metatracheal parenchyma, one cell of the middle row being radially divided into two cells, beneath which was a microscopical globule of excretion in an intercellular space. Another very simple gland in cross-section showed three radial rows of metatracheal parenchyma, the middle row being much narrower than the others, and on each side of one cell in this row a small intercellular space containing excretion. Both these glands were purely schizogenous.

In the pneumatophore of *Carapa* hereafter described more numerous types of schizogenous glands were seen; they are referred to later in this paper.

*Nature of the excretion and of the substance deposited in the wood-vessels.* Before considering the origin and significance of the excretory tissue it is necessary to deal with the nature of the excretory substance itself, and to compare this with the substance present in the vessels of the heart-wood and partly plugging them. In the succeeding remarks the former substance will be termed merely the 'excretion', and the latter the 'plugging substance'.

*Solubility.* Neither swells nor dissolves in water (boiled for three hours), absolute alcohol, xylol, acetone, pure sulphuric acid (cold, 30 minutes), nor concentrated caustic potash (48 hours).

Below are given some reactions of the two substances. The natural colour of the excretion is yellow, that of the plugging substance darker yellow to light brown.

<i>Reagent or Dye.</i>	<i>Excretion.</i>		<i>Plugging Substance.</i>
	<i>Peripheral Part.</i>	<i>Central Part.</i>	
Iodized chloride of zinc, or Vetillart's dilute sulphuric acid and iodine.	Dark orange-yellow.	Brownish yellow to brown at the centre.	Dark yellow to deep brown.
Phloroglucin and hydrochloric acid ( <i>a</i> ) before boiling in water; ( <i>b</i> ) after boiling in water for 3 hours.	( <i>a</i> ) Crimson red. ( <i>b</i> ) Yellowish brown to light pinkish brown. (The cell-walls still stained pink.)	( <i>a</i> ) Reddish orange to yellow. ( <i>b</i> ) Same as periphery.	( <i>a</i> ) Yellow (like the central part of the excretion). ( <i>b</i> ) Yellowish brown to very deep brown.
Ferrous sulphate.	Unchanged.	Dark in the centre and eventually black.	Black.
Methylene blue followed by 2 % acetic acid for 2 days.	Blue.	Blue, sometimes darker than peripheral part.	
Alkannin 50 % alcoholic solution, 24 and 48 hours.	Unchanged.	Unchanged.	Unchanged.
Sudan III for 18 hours, and mounted in glycerine.	Bright yellow.	Bright yellow.	Yellow.
Solution of copper acetate for 15 days.	Orange.	Orange.	Brown.
Pure nitric acid 30 minutes, followed by strong solution of iodine.	Vivid red.	Vivid red.	
Gentian violet (which stains the lignified walls pure blue).	Violet.	Violet.	Light brown with a tinge of lilac.

The excretion in my material was interrupted repeatedly along its length at microscopical intervals by narrow transverse empty spaces, and the two adjoining transverse surfaces bounding a space often conformed in

curvature. My material was well-seasoned wood, and consequently had shrunk. The transverse interruptions may represent ruptures induced by drying and consequent shrinkage of the excretion, associated with the very feeble longitudinal but considerable transverse shrinkage of the enveloping tissue that characterizes all wood. If this be so, the excretion at one time was a gel capable of swelling and shrinking.

The excretion is singly refractive.

The facts recited prove that the excretion and plugging substance give reactions disagreeing with those for cellulose, pectic substances, resin, and swelling gums and mucilages; and that at least the outer part of the excretion contains a substance responsible for lignin reactions; while the central part of the excretion often does not do so, but contains 'tannin' and thus resembles the plugging substance.

The excretion thus agrees with so-called 'wound-gum' that is apt to appear in wood-vessels after the injury of the wood and is of unknown chemical composition. There is consequently no justification for describing the excretory glands as resin-glands or resin-passages.

*Black pigment.* The fact that only in the heart-wood is the excretory band black is due to the deposit of black granules in the parenchyma surrounding or embedded in the excretion: the pigment appears first in the heart-wood.

*CARAPA MOLUCCENSIS*, VAR. *GANGETICA*.

The excretory tissue in the pneumatophores examined, and here described, is less extensive than in *Loooa*, in that an individual band of tissue including glands occupies in cross-section only a relatively short arc: and such appears to be the case in the stem, according to the descriptions supplied by Moeller and Janssonius.

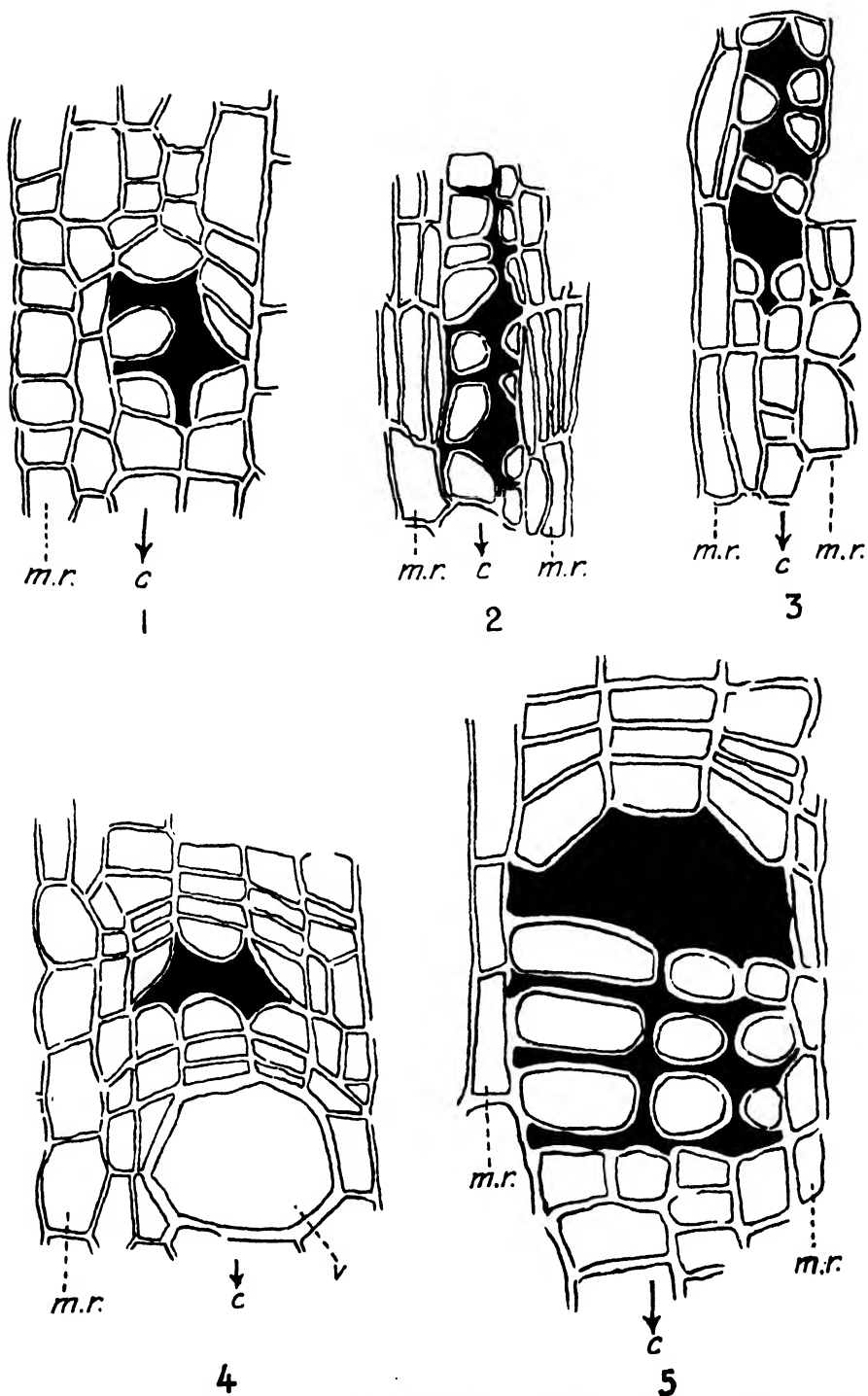
The structure of the wood of the pneumatophore has been described in a previous paper (Groom and Wilson). The glands occur mainly, but not exclusively, in well-developed bands of metatracheal parenchyma, whose cells are ranged in radial series.

The structure of a gland (Pl. XX, Fig. 9) with a large compact mass of lysigenous excretion often agrees with that of *Loooa*. For radially outside and inside the mass of excretion are, in cross-section, shorter or longer radial lines of more or less radially compressed parenchyma cells: these radial lines of cells are often clearly continuous with the radial bands in the excretion that denote immolated lines of cells or are cells traversing the excretion. Round the general mass of lysigenous excretion and continuous with this is excretion in the intercellular spaces among the surrounding parenchyma. Not only compact longitudinal columns of cubical cells, but also cells immediately surrounding the columnar mass, can become con-

verted into excretion, for in radial section a small rounded gland between two closely superposed medullary rays showed at the top and bottom in the excretion a vacuolated pattern clearly marking the converted cells which originally were curved or ran parallel with the ray cells. Moreover, lines of rounded cells traversing the mass of excretion show more or less advanced change into excretion. In addition, round the lysigenous excretion the intercellular spaces contain more or less excretion. Thus the excretion arises in three different ways in these glands: as a schizogenous substance, by conversion of columns of cubical cells, and by conversion of lines of rounded cells traversing the lysigenous mass or of marginal cells encircling this.

Many of the smaller glands, and even some larger ones, are wholly schizogenous. Where such a gland arises in a tangential band of parenchyma, between two neighbouring medullary rays, the parenchyma cells, as seen in transverse section, are at first regularly arranged in radial and tangential lines (though here and there a cell in these lines may divide into two), and a common, if not universal, mode of development of the gland takes place as follows: The excretion is poured into intercellular spaces that arise between the successive tangential layers of cells, so as to cause the gland to consist of alternate tangential uniseriate lines of parenchyma and tangential bands of excretion; but these latter become connected with one another by thicker or thinner radial bands or lines of *intercellular* excretion perforating the tangential rows of cells. The excretion also may also be lodged in the intercellular spaces of the flattened parenchyma cells immediately on the radially inner and outer faces of the glandular tissue described, and here and there may even invade the intercellular spaces between the contiguous cells of the medullary ray. Where the excretion invades the region of the surrounding flattened cells, at least frequently it does not fill the intercellular spaces: it may form a more or less interrupted thin sheet investing each cell; the gaps in the sheet may be very small and in face view resemble smaller or larger pits, while in longitudinal radial section the excretion is visible in the form of a row of very thin spindle-shaped or linear bodies attached to longitudinal cell-walls: in other cases the excretion is more limited and occurs merely in the form of lines running in the fine intercellular spaces at the angles of the flattened cells. Consequently substances can enter and leave these flattened cells without being compelled to pass through the excretion.

The number of tangential and radial lines in transverse section that participate in the construction of a schizogenous gland, or schizogenous part of a gland, varies. Text-figs. 1-5 represent transverse sections of simple glands formed by only two or three radial rows of cells: the glands shown in Text-figs. 1-4 are purely schizogenous, as is probably that shown in Text-fig. 5. But the number of radial and tangential lines of cells con-



TEXT-FIGS. 1-5. Transverse sections of simple glands of *Carapa moluccensis*, var. *gangetica*.  $\times 350$ . The arrows point directly to the organic centre of the pneumatophore. m.r. = medullary ray.

cerned in the transverse section of a schizogenous gland may each reach eight.

Owing to the relatively greater development of the schizogenous part of the gland, *Carapa*, in comparison with *Lovoa*, showed more numerous and distinct transitions between large, mainly lysigenous, and elaborate schizogenous glands. Such schizo-lysigenous glands were apt to give evidence of the relatively late partial conversion of rounded cells embedded in the excretion into additional excretion. For instance, in some cases the gland (in transverse section) included dense masses of excretion of unequal dimensions, and the largest mass always occupied the outer and middle part of the gland (Fig. 9), and often included transitions from rounded cells containing the excretion merely lining a persistent but thin cell-wall to vestigial cells solely represented by vacuole-like structures. Again, in cross-section one crescent-like excretory band, five millimetres in length, showed towards its inner part or throughout its radial thickness glands of the schizogenous type of structure and consisting of alternating tangential uniseriate lines of parenchyma and intercellular sheets of excretion; but locally in the outer part of this band, where a large gland occurred, this type of structure gave way to the lysigenous type and the mass of excretion included remnants of immolated cells. The different staining of the intracellular and intercellular secretion was marked in this case: for with licht-grün the latter stained turquoise-blue, while the former, like the contents of the adjoining tannin-containing parenchyma and ray-cells, stained sap-green. But the presence of tannin in the wood-parenchyma near the glands is not universal; in one case where the tannin was absent from the neighbouring wood-parenchyma (though present in the medullary rays traversing this), the excretion (lysigenous and schizogenous) failed to give with iron salts the reaction for tannin, but throughout assumed a beautiful crimson colour with phloroglucin and hydrochloric acid. So vivid and so much more intense was this crimson colour in the excretion than in the lignified walls of the parenchyma that it was easy to see that the film of intercellular excretion investing a flattened parenchyma cell was quite distinct from the cell-wall. On the other hand, in one section that passed through wound-wood that was gorged with tannin, the plugging substance in the vessels contained no tannin, except where fungal hyphae permeated the vessel.

#### ORIGIN AND SIGNIFICANCE IN *LOVOA* AND *CARAPA*.

Well-developed glandular tissue in *Lovoa* consists of a fenestrated cylinder, or part of one, perforated by medullary rays and here and there interrupted by wood-parenchyma or even vessels. Such tissue is treated here as consisting of a number of glands joined together. In *Carapa*, well-developed glandular tissue is seen in transverse section to form an arc, and



to agree generally with that of *Lovoa*, but it has not been established that this arc represents the cross-section of a network of excretory tissue.

The glands generally arise in the metatracheal bands of parenchyma, but in *Carapa* they sometimes occur in little patches of parenchyma that do not attain the dimensions or form of a distinct tangential band.

As seen in transverse section, the smallest and simplest glands, not nearly occupying the local space between two neighbouring medullary rays, are mainly or possibly exclusively schizogenous, at least in *Carapa*. It was not possible to ascertain whether or no these were isolated glands or prolongations of a more extensive system above or below. Again, where in transverse section an arc of excretory tissue dies out at its margins, the glands situated nearer to these are not only smaller but are also increasingly schizogenous. It therefore appears that the less potent the stimulus tending to evoke the production of glands (or the more powerful the resistance to this) the greater is the tendency for the glands to be schizogenous. It might therefore be anticipated that in *Carapa*, in which the excretory system is much less developed than in *Lovoa*, the schizogenous in comparison with the lysigenous excretion would be relatively more developed than in *Lovoa*: and such appears to be the case.

#### *Origin of the Excretory Tissue.*

Where the glands are purely schizogenous, there is no evidence of any increase in the number of cell-divisions of the metatracheal parenchyma, but where the glands are largely lysigenous there is an increase in such divisions by tangential and transverse, and possibly radial walls; for a lysigenous mass of excretion shows longitudinal columns of short cubical cells converted into excretion, while on the radially outer side and sometimes inner side of the excretion there occur radially arranged, radially compressed cells.

The presence of glands in the sap-wood not far distant from the cambium shows that they can arise early in young wood. The lack of distortion of the tissue surrounding or abutting on a lysigenous gland, as well as the orderly arrangement and shapes of the cells immediately above and below or radially outside the excretion, and of the cells in larger schizogenous tissues, suffice to prove that the gland arises in parenchyma that has just been cut off from the cambium. The gland is consequently to be regarded as the main secondary tissue, and not as tertiary tissue produced by the metamorphosis of mature parenchyma. Where the gland is wholly schizogenous and the excretion merely collects in intercellular spaces among cells not widely different in form from ordinary wood-parenchyma the secondary origin is clear. But there is evidence suggesting that in lysigenous glands some of the cells survived the primary lysigeny, since cells

traversing or bounding the excretion may later in life undergo degradation and thus yield additional excretion: this would approximate to a tertiary origin of portions of the gland.

If the inception of a lysigenous gland follow in its order of development that of normal tissues derived from the cambium, it begins in a schizogenous manner, becomes lysigenous, and ends by the reversion to the schizogenous type; such a scheme would also conform with the conception of a waxing and waning in the tendency to produce excretion. The evidence in favour of this scheme is that the smallest glands in all observed cases were purely schizogenous: that in the highly developed schizo-lysigenous glands of *Carapa*, in a metatracheal band, the schizogenous part of the gland, consisting of alternating tangential layers of cells and excretion, is well developed towards the inner belt of the band. Even if such be the regular centrifugal order of development of the gland tissues, it is probably disturbed by the subsequent excretory degeneration of cells traversing or bounding the lysigenous excretory mass.

#### *Nature and Significance of the Excretion.*

The excretion agrees as regards insolubility and lack of swelling with the plugging substance of the wood-vessels and with wound-gum present in wood-vessels of many dicotyledons; with the latter it agrees wholly or partially in giving the typical reaction of lignified walls with phloroglucin and hydrochloric acid. Both in *Carapa* and *Lovoa* the lysigenous excretion often agrees with the plugging substance in the vessels by containing tannin, which seems to be always absent from the intercellular excretion.

Apart from the fact that the plugging substance in *Lovoa* and *Carapa* show no reaction for 'lignin', this substance differs from the excretion in time of origin as it arises in the old sap-wood when this is changing into heart-wood.

The likeness as regards mode and site of origin existing between, on the one hand, the excretion and, on the other, the plugging substance and wound-gum in wood-vessels becomes very close in the glands of *Cedrela febrifuga* var. *glabrior*, according to the description supplied by Janssonius, who states that in his specimens the zone of glands occupied the place normally taken by a zone of wide vessels forming the pore-zone of a growth-ring (compare annual ring), and he definitely alleges that the gland takes its origin in a vessel. It is, however, not clear if Janssonius means to imply that vessels actually arise and become filled with excretion poured into them by the adjoining parenchyma, or if he means merely that gland tissue arises in spots normally occupied by vessels.<sup>1</sup> In either case the difference

<sup>1</sup> The actual words used by Janssonius are: 'ist noch deutlich zu sehen dass sie [die Sekretlücken] in diesen grössten Gefässen ihren Ursprung nehmen und sich von diesen aus Lysigen erweitern [sic] durch Auflösung des Holzparenchyms, welches hier die Gefässe ganz umgibt.'

between the normal production of plugging substance in wood-vessels and the excretion is that parenchyma in the immediate neighbourhood of the vessels, instead of functioning normally for years, became excretory at a very early stage of its existence, and precociously manufactured a substance corresponding to, or closely resembling, plugging substance or wound-gum. Precocious manufacture of wound-gum and the accumulation of this inside vessels is widespread among woody species when the sap-wood is wounded or invaded by fungi.

Some light on the cause and significance of the origin of the excretion may be thrown by the consideration of other cases of the abnormal production in the secondary xylem of substances that, like this, are not utilized in subsequent metabolism.

The abnormal production of resin-ducts or resin-pockets in secondary wood perhaps presents the strongest analogy, as in this case, too, it is change in the nature of the cells produced by the cambium that is structurally responsible for the abnormal excretory tissue. In *Coniferae* resin-ducts may, as a consequence of wounds, arise in wood that normally includes no resin-ducts, and abnormally large and numerous resin-ducts may be produced as a consequence of fungal attacks; in these cases the ducts may extend to some distance from the causal agency. In *Cedrus* the wood frequently shows, near the outer boundary of the annual ring, arc-like belts of resin-sacs, the cause of the abnormality being unknown, as is also frequently the case when large resin-pockets are produced in the wood of *Picea*, *Pinus*, *Pseudotsuga*, and other *Conifers*. Abnormal resin-sacs, according to Sorauer (p. 709), may arise in *Conifers* without any obvious external cause: for instance, in young plants of *Pinus sylvestris* grown on strongly manured moorland soil. Apparently, then, resinosis (like gummosis in *Dicotyledons*) can be a functional or physiological disease.

In the case of the abnormal production of cells, spaces, or ducts containing resin by the *Coniferae*, it is clear that the stimulus causes the cambium to produce increased amounts of wood-parenchyma, and that this parenchyma manufactures the same kind of excretion as occurs in normal parenchyma of the species elsewhere (cortex, leaf-tissue). *En passant* it may be suggested that the appearance of resin-ducts in traumatic wood of species of *Abies* and *Sequoia*, whose secondary xylem is normally devoid of these, does not in the least imply that this change is a reversion to an ancestral condition: the resin-ducts may be produced in these cases in the wood because they are produced by the plant wherever there is a sufficiently large mass of parenchyma (for instance, in the cortex and leaves of these species).

In *Lovoa* and other species, on the contrary, excretion and glands produced are quite different from the excretion (resin) and excretory cells in the leaves of *Meliaceae*.

Yet between the two cases there are several points of agreement. In the Coniferae, as the sap-wood is converted to heart-wood, the parenchyma of the ray-cells and the genuine tracheides acquire increased quantities of resin, which act in the tracheides as a water-arresting substance; whereas in Dicotyledons plugging substances are produced at the same time in place of resin.

Thus in Coniferae and many Dicotyledons, when the sap-wood is wounded or about to change into heart-wood, the parenchyma is locally moribund and exposed to partial desiccation, and resin or plugging substance is produced. There is, however, no evidence that in *Carapa* or *Lovoa* the glands arise as a consequence of desiccation, wounds, or fungal attack.

*Lovoa* supplied no evidence suggesting any one of these agencies as being a cause of the production of glands. The pneumatophores of *Carapa* were wounded during life and included fungal hyphae, but the latter may have developed after the death of the organ; moreover, previous investigators make no mention of any evidence of fungal action or the presence of wound-tissue.

The sole facts shedding any light on the cause of origin of the glands are those concerning the precise distribution of these. In *Lovoa* the distances apart of the obvious sheets of glandular tissue are so uneven that the phenomenon cannot be a periodic one. This is also proved by the fact that in *Lovoa* three glandular zones may follow one another at merely microscopical distances apart. Again, in *Carapa*, in one case, as seen in cross-section, two short arcs, about 5 mm. in length, were superposed, the outer one being separated from the inner by a distance equal to about twenty parenchyma cells. It is worthy of note that in these cases of glandular arcs occurring successively between the same radii normal metatracheal parenchyma may intervene between the two arcs, so that it is not lack of parenchyma that causes any postponement of the production of the outer glands.

One transverse section of *Carapa* showed two features: first, extreme shortness of some arcs of metatracheal parenchyma occupied by glands, and, secondly, that on the same radii, at minute distances apart, several concentric series of glands can occur. In the transverse section of the wood between two adjoining pairs of medullary rays, in a belt of metatracheal parenchyma (eight cells in radial thickness), were three large glands. A little farther inwards (the distance being equal to about ten wood-parenchyma cells), separated tangentially from the nearest of the three glands before mentioned by only two medullary rays, were two similar large glands between the next two pairs of rays. On the same radii as these five glands, at a distance inwards equal to about forty parenchyma cells, was another gland-containing arc of parenchyma, which bore glands between all the

rays before mentioned and also between the next two rays tangentially on the side of the two outer glands, and between the next five rays (omitting one pair) tangentially on the side of the three outermost glands. These facts prove that, whatever be the cause evoking the production of glands, it is liable to be repeated at longer or shorter intervals, or to continue its action for periods of shorter or longer duration along the same or adjacent radii.

#### SUMMARY.

The excretory tissue of the secondary xylem of the stem of *Lovoa* and pneumatophore of *Carapa*, when well developed, in transverse section forms a thin band parallel to the circumference of the axis: in *Lovoa* the band is long, and may perhaps form a complete circle, and represents a cross-section of a fenestrated cylinder, or part of one, which can extend for at least several feet along the stem and is perforated by medullary rays. Where the belt was smaller, as always the case in *Carapa*, it formed a short arc in transverse section and probably extended down the axis of *Carapa* only a short distance. The excretory tissue is in this paper treated as composed of a number of glands joined together.

The excretory tissue is mainly produced directly by the cambium in place of normal metatracheal parenchyma, and preserves evidence that, like this, its cells were originally arranged in radial (and tangential) sheets.

The smallest glands seemed always to be schizogenous. A typical schizogenous gland in transverse section shows uniseriate tangential rows of parenchyma alternating with tangential bands or masses of excretion, which, however, perforates the sheets of parenchyma by radial extensions, and thus renders the intercellular excretion continuous throughout the gland. This type of gland may be reduced by decrease in the number of tangential and radial rows of cells producing the gland.

In well-developed excretory bands schizo-lysigenous glands occur. Each of these includes a mass of excretion that often preserves in all planes the cellular pattern corresponding to its production by the metamorphosis of longitudinal columns of cubical cells ranged in tangential and radial sheets. The excretion is mainly homogeneous but partly granular, and is traversed by rows or sheets of rounded cells. Some of these, as well as some rounded cells projecting from the outside into the excretion, undergo complete or partial conversion into excretion. On the radially outer, and often inner, sides of the excretion are radial sheets of radially flattened parenchyma, in the intercellular spaces of which excretion occurs in amounts that decrease with increasing distance from the main mass of excretion.

Schizo-lysigenous glands combining the structure of the two types of

glands described in the two immediately preceding paragraphs occur in *Carapa*.

The excretion gives the reactions for wound-gum, and in particular (except sometimes in the centre of a mass) shows the colour reactions (with phloroglucin and hydrochloric acid, &c., and dyes) for 'lignin'. This likeness to wound-gum is enhanced in the case of *Cedrela febrifuga* var. *glabrior*, in which, according to Janssonius, the excretion arises in wide wood-vessels and subsequently increases by lysigenous conversion of the surrounding parenchyma. There is no justification for describing the excretion as resin.

The cause of the production of this excretory tissue is unknown, but the phenomenon recalls certain cases of resinosis in Conifers and gummosis in Dicotyledons. The unevenness of the radial distances apart of the successive excretory bands or arcs proves that the production is not periodic.

For the preparation of microscopical sections used in this work, and for the production of most of the photo-micrographs illustrating this paper, I am indebted respectively to Messrs. G. R. Oliphant and J. M. Branfoot, who were subsidized by the Department of Scientific and Industrial Research.

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#### EXPLANATION OF PLATE XX.

Illustrating Professor Groom's paper on Excretory Systems in the Secondary Xylem of Meliaceae.

c = part directed towards the centre of the stem.

*Louoa Klainiana*.

Fig. 1. Part of large glandular belt in transverse section.  $\times 38$ .

Fig. 2. One gland from the belt shown in Fig. 1, in transverse section, showing the radial pattern of the lysigenous excretion continuous with the radial lines of parenchyma.  $\times 75$ .

Fig. 3. Part of large glandular belt in radial longitudinal section; one gland showing the longitudinal lines in the lysigenous excretion marking the arrangement of the immolated cells.  $\times 38$ .

Fig. 4. Ditto.  $\times 138$ .

Fig. 5. Part of large glandular belt in radial longitudinal section, showing one short gland between two superposed medullary rays, and the other gland tapering as it nears a medullary ray.  $\times 44$  (circa).

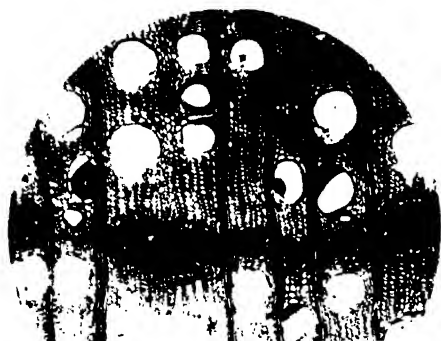
Fig. 6. Part of a feebly developed glandular belt with small glands, in transverse section.  $\times 38$ .

Fig. 7. Ditto, showing in transverse section one gland included in Fig. 6.  $\times 138$ .

Fig. 8. One very small gland between two medullary rays in transverse section, showing excretion in one larger space and in a smaller space interrupting a radial line of cells.  $\times 550$  (circa).

*Carapa moluccensis* var. *gangetica*.

Fig. 9. Part of a glandular belt in transverse section; the excretion is largely schizogenous, but the larger masses are probably at least partly lysigenous.  $\times 156$ .



1.



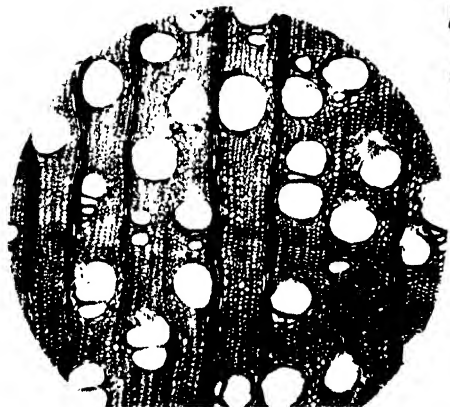
2.



3.

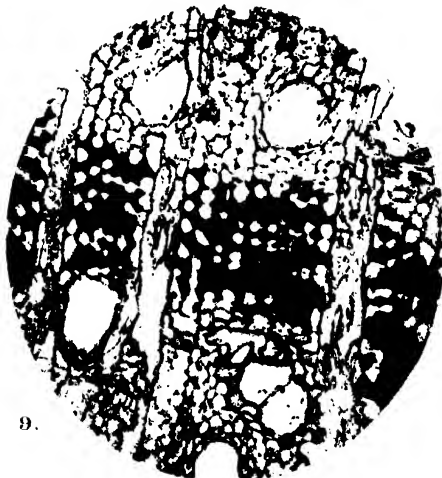
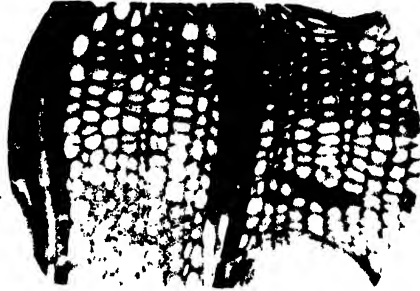


4.



6.

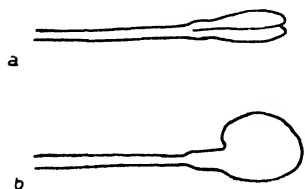






performed. As soon as the cotyledons appeared, the pots were surrounded with cylinders of white paper (as by Bach (1)), so that the illumination was equalized and the hypocotyls grew upright. All experiments were performed on healthy seedlings growing normally during and after the experiments, and no seedlings were used until the cotyledons were unfolded and the hypocotyls upright.

Experimentation has been effected by the upright method, the seedlings being placed horizontally for the desired length of time and then replaced upright. Control experiments, in which the seedlings were rotated on a klinostat after stimulation, showed that there is no difference in the results obtained by the two methods, which is in accordance with Waight's results for fronds of *Asplenium bulbiferum* (9).



TEXT-FIG. 1. To show position of seedlings during stimulation :  
a. in the cotyledonary plane ;  
b. in the intercotyledonary plane.

To prevent sagging during long periods of stimulation, the hypocotyls were supported on white cotton-wool.

The hypocotyl is said to be stimulated in the cotyledonary plane when the hypocotyl and the plane of the cotyledons are horizontal, stimulation being in the intercotyledonary plane when the hypocotyl is horizontal and the plane of the cotyledons is vertical (Text-fig. 1, *a* and *b*).

The presentation time has been taken as that period of stimulation to which at least 75 per cent. of the plants used respond by a curvature of about  $5^\circ$  (rarely exceeding  $10^\circ$ ), the curvatures being measured with a transparent protractor.

The latent time is the period elapsing between the beginning of the stimulus and the first visible movement.

## RESULTS.

### 1. *Experiments to find the Presentation Times for the different Planes of the Hypocotyls of various Seedlings.*

A total of over 900 experiments were performed in this section on the following seedlings :

<i>Lupinus polyphyllus</i>	<i>Ricinus communis</i>
<i>Lupinus albus</i>	<i>Matthiola</i> sp.
<i>Cucurbita Pepo</i>	<i>Solanum Lycopersicum</i>
<i>Helianthus annuus</i>	<i>Sinapis alba</i>
<i>Aquilegia</i> sp.	

Some experiments performed on *Lupinus polyphyllus* are given in Table I as an example of the method by which all the results have

been obtained, and the total results are recorded in tabular form in Table II.

It has been found during the work that there is a definite grand period of irritability to gravity for each species of seedling, and therefore care has been taken to use seedlings during the stage of maximum irritability in each case.<sup>1</sup>

TABLE I.

*Lupinus polyphyllus* :

(a) Stimulated in cotyledonary plane.

<sup>2</sup> Height in cm.	Period of Stimula- tion in Minutes.	Angle of Curva- ture.	Latent Time in Minutes.
2.9	15	—	—
3.4	"	10°	43
3.7	"	—	—
3.8	"	—	—
4.2	"	—	—
5.0	"	—	—
1.5	20	5°	49
2.0	"	5°	49
2.5	"	5°	34
3.0	"	5°	34
4.0	"	10°	60
4.5	"	10°	63

Presentation time = 20 minutes.

(b) Stimulated in intercotyledonary plane.

1.5	20	—	—
2.0	"	—	—
2.5	"	—	—
3.0	"	—	—
4.0	"	—	—
5.0	"	—	—
1.5	60	—	—
1.8	"	—	—
2.2	"	—	—
3.0	"	10°	77
3.5	"	10°	120
6.0	"	—	—
2.2	80	10°	92
3.0	"	10°	143
4.0	"	—	—
4.6	"	10°	86
5.0	"	5°	86
6.4	"	5°	86

Presentation time = 80 minutes.

<sup>1</sup> It is hoped that further work on this subject may be completed at a later date.

<sup>2</sup> The heights of the hypocotyls recorded in the first column of Table I = distance between collet and cotyledonary node.

TABLE II.  
Summary of Results.

P.T. = deduced presentation time. L.T. = average latent time.

	Cotyledonary Plane.			Intercotyledonary Plane		
	Length of Stimulus in Min.	No. used.	Percent-age Response.	Length of Stimulus in Min.	No. used.	Percent-age Response.
<i>Lupinus polyphyllus</i> . Height 1-6 cm.	15	8	12.5	20	21	0
	20	31	87	60	27	44
				75	16	44
				80	24	83
	P.T. = 20 minutes. L.T. = 59 minutes.			P.T. = 80 minutes. L.T. = 103 minutes.		
<i>Cucurbita Pepo</i> . Height 2-12.5 cm.	5	4	0	6	12	0
	6	12	100	12	11	36
				18	18	77.5
	P.T. = 6 minutes. L.T. = 41 minutes.			P.T. = 18 minutes. L.T. = 56 minutes.		
<i>Helianthus annuus</i> . Height over 1 cm.	2	14	50	3	4	21
	3	12	75	4	5	40
				5	8	85
	P.T. = 3 minutes. L.T. = 41 minutes.			P.T. = 5 minutes. L.T. = 56 minutes.		
<i>Aquilegia</i> sp. Height 1.5-2.6 cm.	30	7	71	30	9	50
	35	10	90	35	14	50
	40	4	100	40	9	55
				45	9	100
	P.T. = 35 minutes. L.T. = 48 minutes.			P.T. = 45 minutes. L.T. = 58 minutes.		
<i>Lupinus albus</i> . Height 4-9 cm.	15	8	37.5	15	6	16.6
	20	16	87.5	20	19	68.4
	P.T. = 20 minutes. L.T. = 40 minutes.			P.T. = 20 minutes. L.T. = 40 minutes.		
<i>Matthiola</i> , sp. Height 1.5-4 cm.	15	15	40	20	6	26
	20	8	50	23	8	87.5
	23	7	71.4	25	6	100
	25	2	100			
	P.T. = 23 minutes. L.T. = 30 minutes.			P.T. = 23 minutes. L.T. = 30 minutes.		
<i>Sinapis alba</i> . Height over 2 cm.	15	87	59	15	90	58
	20	108	76.3	20	108	77.7
	P.T. = 20 minutes. L.T. = 40 minutes.			P.T. = 20 minutes. L.T. = 40 minutes.		
<i>Ricinus communis</i> . Height 5.5-21.5 cm.	3	4	25	3	6	0
	5	5	100	5	5	80
	10	3	100	10	5	100
	P.T. = 5 minutes. L.T. = 36 minutes.			P.T. = 5 minutes. L.T. = 36 minutes.		
<i>Solanum Lycopersicum</i> . Height 2-5.5 cm.	5	3	66	5	5	60
	10	24	70.8	10	20	75
	15	14	85.7			
	20	8	75.0			
	30	5	100			
	P.T. = 10 minutes. L.T. = 37 minutes.			P.T. = 10 minutes. L.T. = 37 minutes.		

TABLE III.

*Table of Presentation Times.*

Plant.	Plane of Stimulation.		Ratio.
	Cotyledonary.	Intercotyledonary.	
<i>Lupinus polyphyllus</i>	20 minutes	80 minutes	1 : 4
<i>Cucurbita Pepo</i>	6 "	18 "	1 : 3
<i>Helianthus annuus</i>	3 "	5 "	1 : 1.67
<i>Aquilegia</i> sp.	35 "	45 "	1 : 1.3
<i>Lupinus albus</i>	20 "	20 "	1 : 1
<i>Sinapis alba</i>	20 "	20 "	1 : 1
<i>Matthiola</i> sp.	23 "	23 "	1 : 1
<i>Solanum Lycopersicum</i>	10 "	10 "	1 : 1
<i>Ricinus communis</i>	5 "	5 "	1 : 1

It is believed, as no other record has been found, that this is the first time the presentation times have been found for five out of the nine seedlings mentioned above, namely: *Lupinus polyphyllus*, *Aquilegia* sp., *Matthiola* sp., *Solanum Lycopersicum*, and *Ricinus communis*. Bach (1) records 20 minutes for *Lupinus albus*, which agrees with my result for that plant, but it is remarkable that two species of one genus, namely, *L. albus* and *L. polyphyllus*, should demonstrate such a difference in behaviour, the presentation time for the intercotyledonary plane of *L. polyphyllus* being 80 minutes. A few references to the results of other workers on some of the other seedlings yield some interesting comparisons with the figures quoted in Table III. Fitting (5) has recorded 20 minutes for the presentation time for *Sinapis alba*, which agrees with the time, in Table III, for both planes.

Bach (1), writing in 1907, recorded 6 minutes as the presentation time for *Cucurbita Pepo* hypocotyls, but he regarded a stimulus which would yield 50 per cent. response as the presentation time, and therefore his presentation time does not correspond with the one given above. The length of stimulus, however, which he recorded as yielding over 75 per cent. response, was 10 minutes, which is very nearly the mean between the presentation times, 6 minutes and 18 minutes, I have found for the two planes. Another comparison with Bach's figures shows a similar correspondence with my results. For *Helianthus annuus* he quoted less than 3 minutes as the presentation time, his figures being 72 per cent. response for 3 minutes, 75 per cent. for 4 minutes, and 80 per cent. for 5 minutes. In this case the 4 minutes yielding 75 per cent. response is the exact mean between my two presentation times, 3 minutes and 5 minutes. It is also interesting to note that Fitting (5) records 5-6 minutes as presentation time for *Helianthus annuus* hypocotyls, and Czapek (4) has given 20 minutes as presentation time for the same plant. Bach explains these differences from his results as being due to differences in the tempera-

ture at which each worked. On reference to the original papers I find that there is only a difference of  $5^{\circ}$  C. between the experiments of Czapek and Fitting, the former working at  $25^{\circ}$  C., the latter at  $20^{\circ}$  C. (approx.). As we know that Bach himself worked at  $20^{\circ}$ – $30^{\circ}$  C., I do not understand how the variation in presentation times can be explained by difference in temperature alone. I therefore venture to suggest that Fitting may have stimulated all his seedlings in the same plane, the intercotyledonary, since the time he found agrees with the presentation time I have found for that plane of the hypocotyl. An illustration of the possibility of one worker using his seedlings all in one plane is the plate in Chapman, Cook, and Thompson's paper (3), in which twenty out of twenty-four *Helianthus* seedlings seem to be stimulated in the cotyledonary plane.

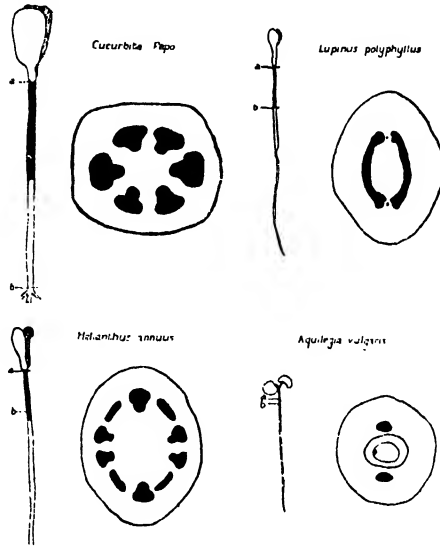
From these results (Table III) the seedlings can be separated into two groups, those which have the same presentation time for both planes of the hypocotyl, i. e. physiologically radial, and those which have different presentation times for the two planes and are physiologically zygomorphic.

Plate XXI illustrates these two groups. Figs. 1 and 2 are photographs of *Ricinus communis* seedlings before and after stimulation, and show equal movement in response to the same stimulus in both planes of the hypocotyl, the seedlings being physiologically radial. Figs. 3–6 are photographs of *Cucurbita Pepo* seedlings which are physiologically zygomorphic. Fig. 3 shows three seedlings before stimulation in two planes of the hypocotyl, to which only those stimulated in the cotyledonary plane respond (Fig. 4). Figs. 5 and 6 show seedlings before and after stimulation for a period, three times as long as that given in Fig. 1, to which all the seedlings respond with curvature in the intercotyledonary plane.

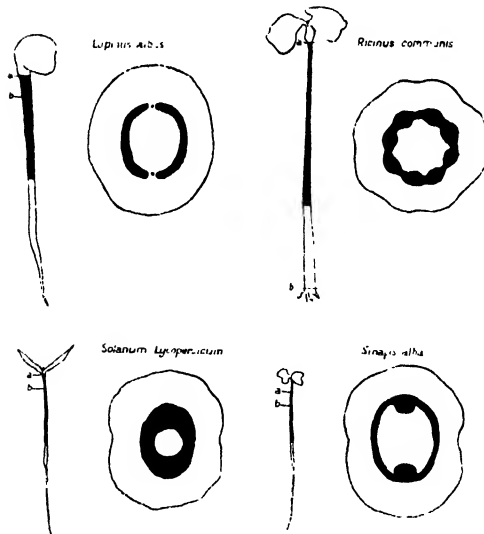
## 2. A Comparison of the External and Internal Morphology of the Seedlings used.

Text-figs. 2 *a* and 2 *b* illustrate the anatomy of the seedlings used. The seedlings are drawn to scale, and it is quite clear that their size and shape have no correspondence with the type of behaviour, as both groups contain large and small seedlings. At a glance it is apparent that there is a striking correlation between the symmetry of structure and the behaviour of the two groups of seedlings, for all in Text-fig. 2 *a* are both structurally and physiologically zygomorphic, and those in Text-fig. 2 *b* are, in general, structurally and physiologically radial. If the part of the hypocotyl which is involved in curvature (marked in black) is compared with the part of the hypocotyl for which the structure is shown (marked *a*, *b*), it will be seen that, of the zygomorphic seedlings, Text-fig. 2 *a*, *Lupinus polyphyllus* and *Cucurbita Pepo* have zygomorphic structure for all the part which bends; *Helianthus annuus* is zygomorphic for the greater portion of the part which bends; and *Aquilegia*, which is least zygomorphic in

behaviour, is zygomorphic in structure for the upper part of the hypocotyl, approximately one half of the part which bends. Of the radial seedlings,



TEXT-FIG. 2 a



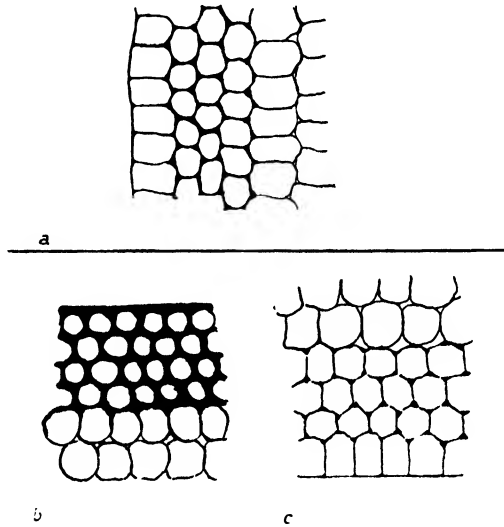
TEXT-FIG. 2 b.

TEXT-FIGS. 2 a and 2 b. Anatomy of seedlings used. The seedlings are drawn to scale, and the part of each hypocotyl marked in black indicates that part of it in which curvature takes place. The structure shown in each section extends over the length of hypocotyl marked a, b. Vascular tissue is marked in black, and the cotyledonary plane is vertical in each case.

Text-fig. 2 b, *Ricinus communis* is radial for the whole length of the hypocotyl and all the part which bends; the other three are elliptical in



cross-section and sub-radial in anatomical structure for the upper part of the hypocotyl, and radial for the greater part of the hypocotyl which is involved in curvature. Previous mention has been made of the different behaviour of the two species of *Lupinus* (Table III), and this is yet more interesting in the light that is thrown on the question by comparison of their anatomical structure. *Lupinus polyphyllus* is elliptical in cross-section and zygomorphic in structure all the way down the hypocotyl, and behaves strongly zygomorphically, whereas *Lupinus albus* is sub-radial in structure



TEXT-FIG. 3. Transverse sections of hypocotyls of *Cucurbita Pepo*. *a*, grown upright—collenchyma normal; *b*, upper side of horizontally grown hypocotyl; *c*, lower side of same.

for a short distance below the cotyledonary node, and is circular in cross-section and radial in structure for the greater part of the hypocotyl, and behaves radially. It therefore seems evident that there is a distinct correlation between the anatomical structure of the part of the hypocotyl in which curvature occurs, and the physiological behaviour of the seedling in response to gravity.

### 3. Experiments on *Lupinus polyphyllus*.

(a) *Behaviour in the dark.* In order to ascertain whether light played any part in the physiological zygomorphy, seedlings of *Lupinus polyphyllus* were stimulated in darkness. Some seedlings were grown in the dark entirely, while others were placed in the dark twelve hours before stimulation. In neither case did the elimination of light reduce the zygomorphic behaviour.

TABLE IV.

*Results of Experiments on L. polyphyllus in the Dark.*

(a) Stimulated in cotyledonary plane.

<i>Length of Stimulus in Min.</i>	<i>No. used.</i>	<i>Percentage Response.</i>
20	8	62.5

(b) Stimulated in intercotyledonary plane.

<i>Length of Stimulus in Min.</i>	<i>No. used.</i>	<i>Percentage Response.</i>
20	7	0
60	8	1.25
75	4	50
80	2	100

(b) *Behaviour of seedlings grown entirely on a revolving klinostat.* *Lupinus polyphyllus* seedlings were grown entirely on a revolving klinostat, so that throughout their entire development the effect of light and gravity was equally distributed.

TABLE V.

*Results on Klinostat.*

(a) Stimulated in cotyledonary plane.

<i>Length of Stimulus in Min.</i>	<i>No. used.</i>	<i>Percentage Response.</i>
15	8	37.5
20	6	100

(b) Stimulated in intercotyledonary plane.

<i>Length of Stimulus in Min.</i>	<i>No. used.</i>	<i>Percentage Response.</i>
20	14	0
40	8	50
50	7	57.5
60	6	83
80	2	100

Presentation time = 20 mins.

Presentation time = 60 mins.

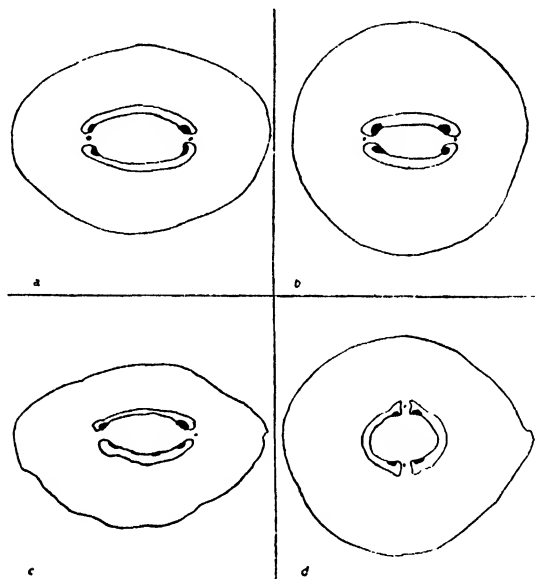
These results show that the growth on the klinostat has reduced the zygomorphy, the ratio for the two planes being 1 : 3 instead of 1 : 4.

Measurements of the cross-sections of hypocotyls grown entirely on a klinostat show that the cross-section is circular, but that the vascular cylinder still retains its zygomorphic character (Fig. 4, b). *Cucurbita Pepo*, when grown on a klinostat, shows no alteration in the shape of the cross-section of the hypocotyl or in the arrangement of the vascular tissue, and also retains the 1 : 3 ratio for the presentation times for the two planes.

## DISCUSSION OF RESULTS.

I have found no previous record of physiological zygomorphy in gravi-perception, but it is a well-known phenomenon in haptotropism and in the movements of tendrils and in pulvini. Haberlandt (6) states that a pulvinus may be anatomically radial, and yet exhibit physiological dorsiventrality, or be both structurally and physiologically dorsiventral.

Peter Stark (8) in his work on the haptotropism of tendrils of *Clematis* found different types of response to haptotropic stimulation, which he considered to be independent of the anatomical structure, since radially constructed tendrils behaved in a strongly physiological dorsiventral manner, and different species of *Clematis*, with similarly constructed tendrils, responded in opposite ways. Thus he concludes that there is possibly some biological explanation of the physiological dorsiventrality



TEXT-FIG. 4. Transverse sections of hypocotyls of *Lupinus polyphyllus*. *a*, grown up-right; *b*, grown entirely on a klinostat; *c*, kept horizontal with intercotyledonary plane vertical; *d*, kept horizontal with cotyledonary plane vertical.

of *Clematis* tendrils, and that the mechanism is such that the most perfect functioning is produced in response to necessity.

On the contrary, in the coleoptiles of Gramineae—which were elliptical in cross-section—he found that the response to haptotropism was dorsiventral in accordance with the anatomical structure, the greater response being in the direction of the broad side and the shorter diameter of the cross-section.

Comparative experiments performed by the writer on the graviperception of *Avena sativa* coleoptiles show a higher percentage of movement, in response to gravity, in the direction of the longer diameter of the cross-section. Although this is contrary to the results found for haptotropism, yet it coincides with the other results for geotropic zygomorphy, for in every case except *Cucurbita Pepo* (Text-fig. 2 *a*) the greater response has been

in the plane of the longer diameter of the cross-section. Stark found that the deviation of the coleoptile cross-section from the radial was proportional to the difference in response for the two planes. A similar relation is evident in the geotropically zygomorphic seedlings, as may be seen from Text-fig. 2. A proof that the difference lies in the power of responsive movement rather than in perception has been effected by giving hypocotyls of *Lupinus polyphyllus* stimuli of twenty minutes in the intercotyledonary plane at 20° C., and placing them at a higher temperature, 30° C., for the latent period. Jost (7) stated that plants stimulated at temperatures at which growth and movement were impossible would respond by curvature when moved to favourable conditions, thus showing that a stimulus was perceived when no movement could result. In the experiments with *L. polyphyllus*, 38 per cent. movement occurred in hypocotyls which were stimulated in the intercotyledonary plane at 20° C. and moved to 30° C. for the latent period, thus indicating a facilitation of the response to a stimulus which was perceived but to which response could not be made at 20° C., when no movement resulted. It therefore seems possible that the zygomorphy may be due to greater difficulty of response to gravity in the intercotyledonary plane than in the cotyledonary plane. This difference in response does seem to be related to the deviation of the cross-section of the hypocotyl from the radial, but that it is not entirely due to the shape of the cross-section, but is also affected by the arrangement of the vascular tissue, is demonstrated by *Lupinus polyphyllus*. When grown entirely on a klinostat, the hypocotyl becomes quite radial in cross-section, but there is still a certain degree of physiological zygomorphy exhibited, the ratio for the two planes being 1 : 3 instead of 1 : 4, which may be due to the retained zygomorphy of the vascular cylinder. It was also mentioned that *Cucurbita Pepo* does not change its shape or exhibit less zygomorphy when grown on a klinostat, and it is significant that this plant differs from the other zygomorphic seedlings by having the greater response in the direction of the short diameter of the cross-section. Hermann Bücher (2) has found variations in the tissues of stems which were laid horizontal and prevented from moving up. Repeating one of his experiments with *Cucurbita Pepo* and *Lupinus polyphyllus*, I have found that *Cucurbita* shows very little alteration in shape when laid horizontal for five days and prevented from curving geotropically. The hypocotyl of *Cucurbita Pepo* possesses a continuous band of collenchyma beneath the epidermis, and the change on geotropic stimulation occurs in this tissue. The upper side of the horizontally placed hypocotyl exhibited greatly thickened walls and smaller lumina in the collenchyma and the lower side was thinner walled than in the normal upright seedling (Text-fig. 3). This explains the lack of change of shape and behaviour when *Cucurbita Pepo* is grown on a klinostat, which equalizes the change in the walls of the collenchyma all round the hypocotyl, making them thinner than in the normal

seedling, but not so thin as the walls of the collenchyma on the lower side of a horizontally laid hypocotyl. If the mechanism of curvature is the same as Bücher (2) suggests for *Ricinus* hypocotyls, namely, compression of the upper side and stretching of the lower side of the collenchyma, it is possible that the wider side would respond more easily than the narrower side of the hypocotyl. *Lupinus polyphyllus* laid horizontally for five days, when the cotyledonary plane was vertical (Text-fig. 4, *d*), exhibited a great change in shape, the parenchymatous cells on the upper side of the cortex being compressed and on the lower side being swollen and causing the cortex to be pushed out of shape. The vascular cylinder was also distorted from the normal and appeared more radial in outline. This shows a greater tendency to change in shape when the cotyledonary plane is vertical, corresponding with the more rapid movement in that plane. When the intercotyledonary plane was vertical for five days, far less change in shape occurred and the vascular cylinder was only slightly distorted (Text-fig. 4, *c*). The resistance to alteration of shape on geotropic stimulation is therefore greater in the intercotyledonary plane than in the cotyledonary, and accordingly resistance to curvature in response to gravity is greater in the intercotyledonary plane. When *L. polyphyllus* is grown on a klinostat, the influence of gravity is equally distributed so that the cells of the cortex are spherical, and the cortex is circular in shape instead of elliptical, but the vascular cylinder retains its zygomorphy, and it seems as if this must account for the retained physiological zygomorphy.

It therefore seems evident that the response to gravity is influenced both by the shape of the cross-section of the hypocotyl and the symmetry of arrangement of the vascular bundles, the stimulus of gravity being followed by a contraction of the cells on the upper side of the hypocotyl and expansion on the lower side, which results in curvature.

In radially constructed hypocotyls, such as *Ricinus*, where the bundles are symmetrically arranged and there is an equal distribution of parenchymatous tissue round them, the response is equal in both planes of the hypocotyl. In zygomorphically constructed hypocotyls the extent of physiological zygomorphy is affected by the degree of structural zygomorphy in the part of the hypocotyl which bends in response to gravitational stimulation.

#### SUMMARY OF RESULTS.

1. Certain seedlings behave in a physiologically zygomorphic manner in response to gravity, which is demonstrated by a difference in the presentation times for the intercotyledonary and cotyledonary planes of the hypocotyl that may be as great as a ratio of 4 : 1 (*Lupinus polyphyllus*).
2. An anatomical investigation of these seedlings indicates the correla-

tion of physiological zygomorphy with anatomical zygomorphy in the part of the hypocotyl which bends in response to gravity.

3. Experiments performed in the dark yield similar results to those in the light, thus eliminating light as a possible factor in this phenomenon.

4. Experiments on *Lupinus polyphyllus* grown entirely on a revolving klinostat show a reduction in the ratio for the presentation times from 4 : 1 to 3 : 1, and an accompanying change in the cross-section, which is radial instead of elliptical. The vascular tissue remains zygomorphic. *Cucurbita Pepo* shows no change in shape or behaviour when grown on a klinostat.

5. By keeping hypocotyls of *Lupinus polyphyllus* horizontal for several days, changes occur in the cortical and vascular tissues which are more marked when the cotyledonary plane is vertical than when the intercotyledonary plane is vertical, thus indicating greater resistance to movement in the latter plane of the hypocotyl.

6. It is therefore probable that response to geotropic stimulation is related to the shape of the cross-section of the hypocotyl and the symmetry of the vascular tissue in that part of it which responds by curvature.

My thanks are due to Professor W. Stiles for allowing me to carry out this research in his laboratory, and also to Miss Prankerd for her kindness in giving me help and advice, without which this work would not have been possible.

UNIVERSITY OF READING.

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## EXPLANATION OF PLATE XXI.

Illustrating Miss Brain's paper on Bilateral Symmetry in the Geotropism of Certain Seedlings.

Figs. 1 and 2 illustrate the behaviour of physiologically radial seedlings of *Ricinus communis*.

Fig. 1. Three seedlings before stimulation. When placed in the horizontal position, the middle seedling received a stimulus in the cotyledonary plane and the other two in the intercotyledonary plane.

Fig. 2. The seedlings of Fig. 1 after placing horizontal; a curvature results from a stimulus in either plane.

Figs. 3-6 illustrate the behaviour of physiologically zygomorphic seedlings of *Cucurbita Pepo*—ratio 1 : 3.

Fig. 3. Three seedlings before a stimulation by which the first two seedlings (from left to right) received a stimulus in the cotyledonary plane and the third in the intercotyledonary plane.

Fig. 4. The responsive curvature in the first two seedlings, stimulated in the cotyledonary plane; no response in the third seedling, which had a stimulus in the intercotyledonary plane.

Fig. 5. Four seedlings before a stimulus.

Fig. 6. The responsive curvature in the four seedlings of Fig. 5 after a stimulus in the intercotyledonary plane, three times as long as the stimulus which produced no response in the third seedling of Fig. 4.



1.



2.



3.



4.



5.



6.

Huth coll.

BRAIN—BILATERAL SYMMETRY IN GEOTROPISM.





# An Investigation into the Cytology and Biology of the Ulvaceae.<sup>1</sup>

BY

NELLIE CARTER, D.Sc.

With Plates XXII and XXIII.

## INTRODUCTION.

IN October 1921 an investigation was started in the laboratory of Professor A. W. Evans at Yale University with the object of getting some knowledge of the cytology and affinities of a somewhat isolated group of the Chlorophyceae, the Ulvaceae. Oltmanns, Wille, Collins, and others believe that this family has some relationship to the Ulotrichales, and by these algologists it is sometimes placed as a constituent family of this group. The late Professor G. S. West, however, supports the view of Blackman and Tansley (3) in listing the Ulvales as a cohort of equal rank with the Protococcales and Ulotrichales. It was hoped that a careful cytological study of some of the members of the family would perhaps throw some light on the relations of this somewhat problematical group. Unfortunately, this object has not been attained with complete success, because in the first place the extremely small size of the nucleus renders an interpretation of the mitotic phenomena extremely difficult, and further we know so little of the cytology of the Ulotrichales that a satisfactory comparison is impossible in the present state of our knowledge. Although the main object of the investigation was not achieved, some interesting peculiarities in the cytology of the algae studied have been discovered, and also some hitherto unobserved facts concerning the sexual reproduction of one of the forms, and it has therefore been deemed well to publish the results of the investigation in their present form.

The writer wishes to take this opportunity of acknowledging the kindness of Dr. Evans, not only for the courtesy extended to her whilst the work was being carried out during the tenure of the Seessel Fellowship at Yale University, but also for his kindness in examining and in helping to inter-

<sup>1</sup> Contribution from Osborn Botanical Laboratory.

pret some of the preparations. Thanks are also due to Dr. G. E. Nichols, who also very kindly gave advice on many occasions, and to Dr. F. E. Fritch for helpful criticism.

#### MATERIAL AND METHODS.

Most of the material used was collected at Fort Hale Park, near New Haven, Conn., on the estuary of the Quinnipiac river. In the fall of 1921 material of *Ulva* was obtained from this locality, and in the early spring of the following year *Monostroma* was collected in abundance. Species of *Enteromorpha* also occurred in some profusion, but material with actively dividing cells was not encountered. Attention was therefore devoted almost entirely to *Ulva lactuca*, Linn., and to two species of *Monostroma*, *M. latissimum*, (Kütz.) Wittr. and *M. Grevillei*, (Thur.) Wittr., var. *Vahlü*, (J. Ag.) Rosenv.

For the purpose of obtaining mitotic figures the material was fixed at intervals of 30–60 min. during night-time. The most abundant mitotic figures were observed in material fixed from 11.30 p.m. to 3.30 a.m.

In the case of *Ulva*, pieces of the frond were fixed in Flemming's weaker solution, made up in sea-water, for about twelve hours or overnight. After a few preliminary rinsings in sea-water, the material was washed for about twenty-four hours in running tap-water. The stain used was Heidenhain's iron-haematoxylin, sometimes with orange G used as a counterstain. Although the thallus is only two layers of cells in thickness it was found necessary to cut sections, owing to the dense nature of the walls.<sup>1</sup> After embedding in paraffin, sections were cut as far as possible parallel to the surface of the thallus. When dehydrated the alga becomes very brittle, and considerable difficulty was experienced in the manipulation of the material after this stage. However, because of this brittleness, whole mounts were often found to be useful, since the fragments of the alga often split between the two layers of the thallus for a small region of the margin, so that many details could be observed in the one-celled layer thus resulting, which would be quite invisible in the normal two-celled layer.

The manipulation of the one-layered fronds of *Monostroma* was even more difficult. The cell-wall is not of the same tough nature as in *Ulva*, so that fixing for twelve hours results in the disintegration of the thallus into very minute fragments, which renders the subsequent washing very difficult. In the later part of the work the plants were only fixed for one hour, and were usually left for several hours in the dilute fluid which results from

<sup>1</sup> The cell-walls in *Ulva* are often quite yellowish in colour, and when tested with potassium ferrocyanide show the presence of traces of iron. Even the delicate walls of *Monostroma* also contain a little iron. The wall, staining very deeply with the haematoxylin by virtue of its iron content, usually obscures the internal structure.

replacing the fixing solution with clean sea-water. After this the material was washed in the usual way. Even with this small period of immersion in the fixing fluid, the plants were reduced in the treatment which necessarily followed to small pieces a few millimetres square at the most, which could not, because of their delicate mucilaginous nature, be readily handled with forceps. In order to reduce manipulation as far as possible, mounts were made in a somewhat rough fashion. Slides were smeared very lightly with Meyer's egg albumin fixative as for paraffin sections, and then flooded with water. In this film of water were floated pieces of the *Monostroma* thallus already fixed and washed, using needles to smooth out the tiny fragments so that they should neither be folded nor overlap, and spreading, as far as possible, just one layer of cells over the slide. After draining off the surplus water the slides were allowed to dry thoroughly, and were hereafter treated exactly as sections on the slide. This seems to be a very crude method, but by allowing the alga to dry on the slide in this way, the cells themselves did not shrink, contraction taking place mostly in the gelatinous matrix in which they are embedded. It was found that these preparations yielded far better results than paraffin sections or than mounts made from material brought without drying into xylol and then balsam before spreading on the slide. It is very difficult to spread out the delicate sheets of *Monostroma* when in the viscous balsam, and such preparations are consequently unsatisfactory.

#### GENERAL STRUCTURE AND CYTOLOGY.

In *Ulva lactuca* the vegetative cells of the thallus are mostly  $7-13\ \mu$  by  $7-11\ \mu$ . Examined from the surface of the frond, they are rectangular to polygonal in shape with somewhat rounded angles (Pl. XXII, Figs. 1, 2, 22) and, as is well known, are arranged in an even sheet two cells in thickness, each layer of cells being about  $15\ \mu$  thick.

Practically all the cells of the thallus are similar to each other, the only division of labour being in the formation of a short stalk and holdfast. The way in which all the cells of the lower part of the thallus contribute to the formation of this holdfast, by sending down slender filaments between the other cells, ending with a dilated extremity in the holdfast, has been investigated by Delf (7). Dr. Delf found that these filamentous cells become multinucleate in *Ulva*. During the investigation of *Monostroma Grevillei*, var. *Vahllei*, the writer has noticed, in contrast, that the cells forming the holdfast remain uninucleate. The nucleus in such cells was always located in the slightly swollen extremity of the filamentous outgrowth, and, because of the great length of the latter, was often considerably removed from the main body of the cell with its chloroplast (Pl. XXII, Fig. 26, n).

In the ordinary vegetative cells the protoplasmic contents are confined

to the peripheral region, the interior of the cell being occupied by a vacuole of considerable size. There is a fairly large platelike chloroplast in each cell, which is disposed under normal conditions on that side of the cell which is exposed to the exterior.

There is usually one pyrenoid in the centre of the chloroplast, but not rarely two are present, and less often three or four (Pl. XXII, Fig. 22). The nucleus is usually disposed in the lining layer of protoplasm, on the innermost wall, exactly opposite to the chloroplast.

A rather striking peculiarity of the cells of *Ulva* is the frequent presence of a distinct flattened platelike area of protoplasm which, when it occurs, always lies in close proximity to the nucleus. This body is seen in Pl. XXII, Figs. 1, 19, 5. It is a polygonal or circular structure connected to the peripheral cytoplasm by radiating strands of protoplasm, and usually lies on the innermost wall of the cell or near to the nucleus. It stains somewhat darker than the rest of the cytoplasm, and its margins are often somewhat emphasized; otherwise it is apparently homogeneous. Exactly what is the nature of this structure is unknown. It was not always visible, but was usually very conspicuous in *Ulva*, although it was not observed in either species of *Monostroma*. All attempts to link up this peculiar body with the centrosomes or similar bodies previously described for algal cells have failed. It does not seem to become conspicuous at nuclear division or behave at all like a centrosome. Possibly it is merely part of the ordinary cytoplasm, which happens to have this unusual orientation in some cells (see addendum).

The structure of the cell in *Monostroma* is essentially similar in all characters to that of *Ulva* (Pl. XXII, Fig. 27; Pl. XXIII, Fig. 28). The thallus is one cell thick, and the chloroplast in all the cells normally occupies the corresponding outer wall, the nucleus being located on the opposite side. Thus in any thallus, on focusing on to the one surface of the frond, either all the chloroplasts with their pyrenoids are brought into view or else all the nuclei on the opposite side of the frond.

#### *The Pyrenoid.*

In all three forms studied there is typically one pyrenoid in each cell, but not infrequently several pyrenoids may be present. The behaviour of pyrenoids during cell-division is a very controversial point. Recent research in various algae tends to indicate that these organs do not follow any fixed rule, but that in different algae they behave in different ways.

Smith (25; p. 463), working on *Characium Sieboldii*, states that during cleavage of the protoplasm in preparation for zoogonidia formation, it is a matter of chance whether or not the segments cut off contain pyrenoids. Some segments may contain two pyrenoids, others none at all. The pyrenoids remained unchanged during the process, being intact with their

starch sheath, according to Smith's statement, until cleavage was far advanced, when they disappeared mysteriously. Sometimes they were visible for a long time between the uninucleate protoplasts, but finally they always disappeared. The young zoogonidia were provided with a new small pyrenoid during the rounding-up process of the separate protoplasts before the release of the zoogonidia. Smith was unable to account satisfactorily for the sudden disappearance of the large unchanged pyrenoids of the zoogonidangium. A similar kind of pyrenoid behaviour obtains in *Scenedesmus*, according to the same author, and also in *Palmella*, according to McAllister (17). In the zoospore formation in *Hydrodictyon* (31), *Cladophora* (28), and during the spermatogenesis of *Sphacroplea*, the pyrenoids disappear somewhat earlier in the process, and in *Pediastrum* (26) they rarely remain until cleavage has begun. In *Pediastrum* Smith was unable to say at what precise step the pyrenoids reappear in the young cells. In *Tetradesmus* (23) the pyrenoid remains unchanged in one of the daughter-cells, disappearing by solution at the end of the second cleavage. A small pyrenoid is formed *de novo* on the completion of the formation of four daughter-cells.

The present author (5), on the other hand, has actually watched the division of the pyrenoid in living cells during cell-division in some Desmids, a fact which tends to show that in this family, at any rate, the formation of pyrenoids *de novo* is not the rule. It should be remembered, however, that conditions are naturally somewhat different in the Conjugatae, for here we do not get anything comparable to the formation of numerous small protoplasts simultaneously from a single mother-cell, as happens so frequently in the Protococcales, and perhaps it is not surprising that the pyrenoid is able to undergo leisurely fission as the cells divide. Haase (12) also describes the division of the pyrenoid during cell-division in *Ulothrix subtilis*, the pyrenoid dividing simultaneously with the nucleus.

Hartmann (13, 14), working with *Chlorogonium elongatum*, Dang., and *Eudorina elegans*, Ehr., found rather diverse behaviour of the pyrenoids in these two forms. In the first species the pyrenoid begins to change at the first onset of nuclear division, being gradually dissolved, although leaving for a time several darkly staining portions. When the nucleus is still in the early prophase of division the two pyrenoids have entirely gone. They reappear in the young daughter-cells at the completion of the last mitosis, and Hartmann was unable to give any suggestion as to their formation. Staining with mitochondrial stains gave no results. Goroschankin (10) has made similar observations on *Chlamydomonas Braunii*. The pyrenoids seemed to disappear during vegetative mitosis. In *Eudorina elegans* (14), however, the facts seemed to be different. In a certain strain normally having one pyrenoid in a cell, this was observed to divide regularly at each division of the chromatophore. In another form having

several pyrenoids, these were distributed amongst the daughter-cells, so that when the young colonies were fully formed, each cell eventually possessed one pyrenoid. In the later development of such a cell multiplication of the pyrenoid occurred often until thirty-two were formed.

Grove (11), on the other hand, not finding any visible pyrenoids in the young daughter colonies of the same alga, concludes that they must be formed *de novo*.

Similar observations to those of Grove on *Eudorina* were made by Zimmermann (32) on *Volvox*. Zimmermann came to the conclusion that increase in the number of pyrenoids in the cells developing into gonidia is not due to division of the original pyrenoid, but to formation *de novo*.

In spite of the exceptions quoted above, it is still a general rule that in the majority of the Chlorophyceae which produce several zoogonidia or other similar reproductive bodies at a time in a mother-cell, sooner or later the pyrenoid or pyrenoids of the original mother-cell disappear suddenly, and with a mystery which never fails to baffle the investigator, only to reappear with equal mystery in the young protoplasts when they are fully formed and ready to begin their independent existence.

In view of this lack of uniformity in pyrenoid behaviour, the pyrenoids were very closely studied during the present investigation, in order to obtain, if possible, some information concerning them. As would be expected, the mother-cells were observed to lose their pyrenoid at some stage or other during the formation of the zoogonidia, a small pyrenoid reappearing in the young zoogonidia before their release. The disappearance of the original pyrenoid, however, was neither as sudden nor as mysterious as described by other investigators, and it was possible very often to trace the pyrenoids for a little while after they had begun to disappear. In the three algae studied it seemed (from the methods used) that two distinct processes could be observed to take place during the disappearance of the pyrenoid, the first being of the nature of a solution process, and the second a fragmentation of the pyreno-crystal, with dispersion of the fragments throughout the chloroplast. There seems to be no hard and fast rule as to which process shall take the greater part in the final disappearance of the pyrenoid in any species. Indeed, both have been observed in parts of the same thallus, and it is suspected that both may occur in the same cell at one time.

The solution process was observed best in *Monostroma Grevillei*. Here, during the whole process of nuclear division, the pyreno-crystal, which in resting cells stains intensely black, loses its staining capacity, and by the time the nucleus has reached the metaphase of division, stains very faintly grey, but is still distinctly visible. In this condition the pyreno-

crystal appears homogeneous and structureless; it is about as large as an ordinary pyrenoid, but is only distinguished from the cytoplasm by reason of its non-reticular appearance (Pl. XXIII, Fig. 34). This gradual waning in staining capacity of the pyreno-crystal suggests that the latter may consist of a fundamental protoplasmic foundation in which are laid down food reserves of a nitrogenous nature, which can be removed or added to, upon occasion, probably by the agency of enzymes. Sometimes a little dark spot may be seen in the middle of the paler mass (Pl. XXII, Fig. 27, *c'*); this may represent an intermediate stage where the solution of the nutrient substances is not quite complete. Not infrequently, previous to this solution process, the pyreno-crystal may show signs of acute fragmentation, but the nearly colourless pyreno-crystal left at the end of the solution process may still be fairly large and intact, though somewhat cracked with fissures (Pl. XXII, Fig. 27, *d*). In newly separated daughter-cells both frequently possessed such faintly staining pyrenoids, but usually one was distinctly darker than the other, although staining by no means as intensely as the ordinary pyrenoid of the mature resting cell (Pl. XXII, Fig. 27, *a, a', b, c, c'*). The inference is that the darker pyrenoid is the original pyrenoid of the mature resting cell, which is bequeathed to one of the daughter-cells, and is beginning to acquire its nitrogenous reserves again, whilst the paler pyreno-crystal of the other daughter-cell is formed *de novo*, possibly from the substances dispersed in the solution of the original pyrenoid. The bequeathed pyrenoid will naturally be darker in staining capacity and richer in food reserves than the newly formed pyrenoid, because it has never actually disappeared. In other cases not one faintly staining pyrenoid was observed in the daughter-cells, but many small ones in one of them (Pl. XXII, Fig. 27, *d, c, f, g, h*). This was particularly the case in *Monostroma Grevillei*, and is probably to be correlated with the frequent occurrence in mature cells of this species of a number of pyrenoids rather than a single one.

The second type of pyrenoid digestion seems to consist of the breaking-up of the pyreno-crystal by means of cracks which chip off tiny pieces. This process is accompanied by the simultaneous appearance of small angular darkly staining bodies in the general chloroplast (Pl. XXII, Figs. 2, 25). It is concluded that these small dark bodies are broken off from the main pyrenoid and dispersed. *Ulva lactuca* showed this phenomenon very well. The fragmentation is usually well advanced by the time the nucleus is in the prophases of division, but it rarely proceeds so far that the pyrenoid entirely disappears. On the other hand, it can usually be seen that the remains of this body is destined to function as the pyrenoid of one of the daughter-cells (Pl. XXII, Fig. 20, *a*). At the meta- and anaphases of nuclear division the pyrenoid is less than one-fourth its usual size, but it is quite homogeneous in appearance. At the time of the telophase it is frequently larger and more distinct, and is now apparently becoming stored with food



substances again, and is taking on the characters of a permanent pyrenoid for one of the daughter-cells. Even after cleavage has begun there is frequently no sign of the appearance of a second pyrenoid for the other daughter-cell (Pl. XXII, Fig. 22, *a*), and even when cleavage (which begins at the opposite end of the cell near the nucleus, and proceeds towards the region of the pyrenoid) is quite complete, one of the daughter-cells may still show no sign of a developing pyrenoid. The darkly staining granules, however, which appeared in the chloroplast on the disintegration of the original pyrenoid are still conspicuous (Pl. XXII, Fig. 20, *a*), and it is not long now before the second cell also has acquired a pyrenoid. Before the cell-wall dividing the two daughter-cells is formed, both have, as a rule, a fully developed pyrenoid. The development of the second pyrenoid can be traced to a slight extent, since it is often seen to be much smaller than that of the sister-cell (Pl. XXII, Fig. 23, *a*). Presumably it is developed from one of the above-mentioned granules which appear in the chloroplast when the pyrenoid of the original cell begins fragmenting. It seems logical to assume that the new pyrenoid of the one daughter-cell arises from one of the fragments dispersed from the original pyrenoid. As a rule only one of the fragments develops at the expense of all the others, but occasionally several develop and a group of pyrenoids results. Even if the original cell should possess two pyrenoids to begin with, it does not follow that one of these will go to each of the daughter-cells. Both will go to the same daughter-cell if they happen to lie on the same side of the cleavage plane (Pl. XXII, Figs. 22, *a*, 28, *a*).

The process of pyrenoid solution in *Monostroma latissimum* was chiefly that of fragmentation (Pl. XXII, Figs. 31, 39), but it must be emphasized that neither type is confined to any one of the three species. In fact parts of the same thallus, and even adjacent cells, may show indications of both processes, whilst, as we have already seen in *Monostroma Grevillei*, the solution process is also accompanied by slight fragmentation of the pyrenocrystal, though without dispersion of the fragments.

Smith (24) describes at some length the disappearance of the pyrenoids in *Scenedesmus* spp. during autocolony formation. He notices a loss in staining capacity, and suggests that enzyme action is probably responsible for the digestion of the pyrenocrystal. Finally, he notices a cavity left where the pyrenoid was originally located, although later this cavity also disappears so that it is impossible to say where the pyrenoid was. Each cell of the new colony acquires a pyrenoid, formed *de novo*. Smith's Figs. 12 and 13 on Pl. XVI and Figs. 55 and 56 on Pl. XVII are very similar to stages in the disappearance of pyrenoids seen in the Ulvaceae, but the idea of the pyrenoid being actually dissolved away and leaving a cavity cannot be supported from the evidence obtained in the present investigation, for in whatever way the pyrenoid was changed, it never actually disappeared. As far as I have been able to ascertain,

no one has previously observed such pronounced fragmentation of the pyrenoid with dispersion of the minute fragments into the general chloroplast as was observed in *Ulva lactuca*, where it was certainly a very conspicuous process. Hartmann's figures (13, Pl. III, Fig. 42) give some slight indication of fragments being broken off from the pyrenocrystal, but he makes no comment on the subject, and they do not suggest such a wide dispersal of the fragments as observed in *Ulva*. In his later work (14, Pl. II, Figs. 13-17 and 19) he figures numerous small dark fragments in the chloroplast, but again as far as I could make out he does not explain their origin, and we cannot assume that he believed them to have any relation to the pyrenoid since he states that the pyrenoids either divide before cleavage of the protoplasm, or, in forms possessing many pyrenoids, that they are distributed one to each of the daughter-cells.

Another investigator who has figured darkly staining granules in dividing algal cells is McAllister (17). Some of this worker's figures (Figs. 7-9, 16, 24, 26, Pl. LVI) show dark particles distributed in the cytoplasm similar to those observed in *Ulva* and *Monostroma* during the present work. Since McAllister gives no explanation of the granules he figures, we cannot be sure that they had their origin in the pyrenoid.

The behaviour of the pyrenoid during vegetative cell-division, therefore, presents some peculiarities in the Ulvaceae, which may be briefly described as follows: sometimes there seems to be a simple solution process, in which the pyreno-crystal gradually loses its staining properties, presumably by the solution of its nitrogenous reserves, without decreasing perceptibly in size, and without the accompanying discharge of particles into the chloroplast as in the second type of pyrenoid behaviour. After the completion of the cell-division and the cleavage of the protoplast, the feebly staining pyreno-crystal regains its staining properties and becomes the functional pyrenoid of one of the daughter-cells. The second daughter-cell acquires a similar pyrenoid or sometimes a group of pyrenoids, staining very faintly at first, but gradually increasing in staining properties. The first origin of the pyrenoid of the second daughter-cell could not be observed directly, but it seemed to arise from a sort of primordium, which stains very feebly at first. In the second type of pyrenoid behaviour there is a fragmentation of the pyreno-crystal, small particles being distributed in the general chloroplast, possibly after partial solution of their contained food reserve. In this process a small portion of the original pyrenoid remains; this, later, serves as the pyrenoid of one daughter-cell. The second daughter-cell develops an independent pyrenoid or sometimes a group of pyrenoids, presumably from the reorganization of one or more of the fragments split off and dispersed from the original pyrenoid. It is probable that the solution process accompanies the fragmentation process to some extent. The first process was observed best in *Monostroma Grevillei*, and

the second in *Ulva lactuca*, but there is no constancy for either process within any species.

Apart from the phenomena described above the pyrenoids were sometimes observed in the process of budding off smaller pyrenoids, or even of dividing more or less equally into two halves in mature cells. The accumulation of too great a food reserve probably induces pyrenoid division in such cases.

In the formation of swarm-spores the behaviour of the pyrenoids was observed best in *Monostroma latissimum*. Here, previous to the first mitosis of the nucleus, the pyreno-crystal fragments and the particles are distributed in the chloroplast as described above for vegetative mitosis. After the first mitosis the pyrenoid material seems to become aggregated again, and collects to form separate pyrenoids, one for each daughter segment (Pl. XXIII, Fig. 43). At the prophase of the second mitosis, fragmentation occurs again (Pl. XXIII, Fig. 44), and the process goes on as before, the pyrenoids becoming reorganized at the close of the telophase (Pl. XXIII, Fig. 48). Thus, after each mitosis, it seems possible for the pyrenoids to become reorganized, and accordingly 2, 4, 8, 16, &c., pyrenoids may be distinguished in the mother-cell as the 2, 4, 8, 16, &c., celled stages of gamete formation are completed. (Cf. Pl. XXIII, Fig. 52, 8-celled stage.) Whether this invariably happens is not absolutely certain, but it was usually so in *M. latissimum* and also in the zoospore formation of *Ulva lactuca* as observed in this investigation. On at least one occasion, however, fragmentation of the pyrenoid did not occur, for Pl. XXIII, Fig. 51 shows a 4-celled stage in late anaphase with the original pyrenoid still intact, although very faint. Evidently, then, the pyrenoid behaves during the formation of the swarm-spores as in vegetative cell-division. It may undergo gradual solution, or it may become fragmented and dispersed through the cytoplasm as tiny granules.

It has occurred to the writer that possibly the reorganization of the pyrenoids at every division of the nucleus may not be normal, since the plants were not fixed until from 10 to 18 hours after being removed from the shore. In the ordinary course of events the completion of nuclear division in the ripe zoogonidium marks the reappearance of the pyrenoids. Perhaps abnormal conditions cause a slowing down of all vital phenomena, including mitosis, which has a similar effect in causing the reappearance of pyrenoids in the plants investigated. It is possible that in normal conditions the organization of pyrenoids would be delayed until the end of the last mitosis.

During the investigation the author was impressed by the periodic loss of staining power by both the nucleus and pyrenoid. In the formation of zoogonidia and gametes, nuclei and pyrenoids were rarely both distinctly visible at the same time; Pl. XXII, Fig. 24 represents one of the few excep-

tions to this statement. This was not true for the first two or three mitoses, when both organs might be readily distinguished (Pl. XXIII, Fig. 48), but later, when both nuclei and pyrenoids were getting exceedingly minute, it was a fact which caused some confusion. When the nuclei were in their most conspicuous stages, i.e. during the ana- and metaphases of division, the pyrenoids were never in a visible condition (Pl. XXIII, Figs. 50, 53-56). During the later stages and in the short period of rest before the succeeding division, however, the nuclei seemed to lose their staining power, being only very slightly darker than the general cytoplasm with Heidenhain's haematoxylin. At this stage the pyrenoids become very conspicuous, staining deep black (Pl. XXIII, Fig. 52). It is not always easy to know whether one has a nucleus or a pyrenoid under observation, but after a little practice it is possible to distinguish between them, since the pyrenoid is always larger and often shows signs of fragmentation. In the later stages of swarm-spore formation, however, the presence of numerous small dark granules, which may be either nuclei or pyrenoids, is very confusing, especially in *Monostroma latissimum*.

#### *The Nucleus.*

The resting nucleus in all three species examined is exceedingly minute. Very often it seems impossible to recognize more than the darkly staining nucleolus, but, after careful examination, it is usually possible to distinguish that this dark spot is surrounded by a narrow dull grey region, which marks the limits of the nucleus proper (Pl. XXII, Figs. 22, 27, *c, j*; Pl. XXIII, Fig. 28, *b, c*). In *Ulva lactuca* the nucleus is somewhat less than  $2\ \mu$  in diameter, and the nucleolus about  $0.8\ \mu$ , whilst in *Monostroma* (both species) it is rather smaller, the entire nucleus being rarely larger than  $1.5\ \mu$  and the nucleolus about  $0.3\ \mu$ - $0.5\ \mu$ . The process of nuclear division was very difficult to follow in structures of such small size, and, although abundant mitotic figures were present, it was very difficult to interpret them.

At the first onset of mitosis the nucleus increases visibly in size, becoming about  $3.5$ - $4\ \mu$  in diameter in *Ulva* and somewhat less in *Monostroma*. This increase in size is accompanied by the appearance of small darkly staining granules in the grey peripheral region of the nucleus which had previously been apparently homogeneous (Pl. XXII, Fig. 1; Pl. XXIII, Fig. 29). Occasionally the appearance of the darkly staining granules of chromatin seemed to be preceded by that of a number of definite rounded bodies, staining rather a darker grey than the general peripheral region of the nucleus, and yet by no means as darkly as the later developed chromatin (Pl. XXII, Fig. 3). As these dark granules become larger and tend to run together, the nucleolus gradually disappears (Pl. XXIII, Fig. 28, *d, e*). It does not seem to contribute directly to the formation of the chromosomes, but when the chromatin is aggregated into distinct long threads, what is apparently the remnants of the nucleolus can be seen in close proximity. Quite

frequently the chromatin becomes arranged in the form of an elongated filament, or several such filaments, not very compact, and sometimes with a larger granule which is probably the remains of the nucleolus (Pl. XXIII, Fig. 30, *n.*?). Sometimes, also, the filament or spireme seems to be segmented, and has the appearance of chromosomes arranged in a linear series. This moniliform spireme was very frequent in *Ulva* (Pl. XXII, Figs. 4, 5); in *Monostroma* the spireme filament was not so regular (Pl. XXIII, Figs. 30–32). Quite possibly these beadlike segments actually represent chromosomes. In a few cases there was indication of the longitudinal splitting of the thread (Pl. XXII, Fig. 6; Pl. XXIII, Fig. 34), but very few examples of this were encountered, and these were found chiefly in the two species of *Monostroma*. Meta- and anaphases were very commonly observed (Pl. XXII, Figs. 7–11; Pl. XXIII, Figs. 35–38), but it was exceedingly difficult to find any definite spindle fibres, or indeed any indication whatever of a definite spindle. The chromatin usually seemed to lie as deeply staining masses in a colourless zone. The stage which the mitosis has attained can be judged by the distribution of the chromatin granules or masses. It is by no means intended to suggest, however, that a spindle is never formed, for, indeed, a definite spindle was observed on about three occasions (Pl. XXIII, Figs. 35, 36). The reason for this scarcity of spindles, in spite of the abundance of the requisite stages of mitosis in the material, is possibly that the spindle fibres possess peculiar staining properties. The minute size of the nucleus also renders observation more difficult. The spindles found were  $5.5\text{ }\mu$ – $7.5\text{ }\mu$  long. The chromosomes were very rarely visible as individual granules in the subsequent stages of division, but usually appeared as a coalesced mass, sometimes constricted at regular intervals as if the chromosomes were closely pressed together (Pl. XXII, Figs. 10, 11; Pl. XXIII, Fig. 37). Most frequently in the lateral view of the metaphase the chromatin appears as a solid bar, the individual chromosomes being indistinguishable (Pl. XXII, Fig. 7). Not infrequently, in the anaphase also, the chromosomes are still seen as a continuous rod of material. Sometimes, however, there is, as before, some indication of constriction at intervals into separate chromosomes, and again the chromosomes may appear to be practically distinct from each other (Pl. XXII, Figs. 12–15).

The actual number of chromosomes present was very difficult to determine, but all things seem to indicate that ten is the number involved (Pl. XXII, Figs. 8, 9). The extremely small size of the chromosomes, and the fact that in so many cases they seem to coalesce, makes it very difficult and usually impossible to count them. In what is apparently the polar view of the metaphase, however, about ten chromosomes can be counted in cells of *Ulva*. The same was often true for the corresponding stage in *Monostroma latissimum*. In *M. Grevillei*, however, eight seemed to be the number indicated, but, since this species was extremely difficult to study, it is quite possible that this is not an accurate count. In the anaphase it was very difficult to determine the number of chromosomes. In *Ulva* it appeared

that certainly not more than five or six chromosomes pass to each pole (Pl. XXII, Figs. 13-15). In *M. Grevillei* the corresponding number four seemed to be confirmed. *M. latissimum*, however, was rather difficult to interpret. Occasionally, the number five was visible (Pl. XXIII, Figs. 37, 39), but, especially in the later mitoses preparatory to zoogonidia formation, there was some indication of two groups of ten chromosomes separating at the anaphase (Pl. XXIII, Figs. 46, 53). The small size of the chromosomes, however, and the absence of spindle fibres made it difficult to determine the actual number of chromosomes and the exact stage of the process under observation. It is possible that the stage represented in Pl. XXIII, Fig. 46 is actually the metaphase of the third mitosis, and not the anaphase of the second. But we should have to assume, on this last supposition, that the cleavage of the protoplast has been delayed, a condition which, although not common, does sometimes occur (Pl. XXIII, Fig. 49). The actual number of chromosomes must therefore be considered doubtful, since it is difficult to decide whether five or ten is the true number for *Ulva*. That ten is the number would fit in very well with the peculiar moniliform spireme so often seen in *Ulva*, yet other evidence points to the fact that five is the true number. Unfortunately, the small size of the nucleus renders a decision uncertain, and, as will be explained later, it is possible that fusion or cohesion of the chromosomes causes further complications, as suggested by Hartmann for *Chlorogonium*.

The telophase of division seemed to present no peculiarities. The chromosomes contract to form a compact mass (Pl. XXII, Fig. 15), whilst later a distinct nucleolus is visible with granules easily seen in the peripheral region of the nucleus, as in the early prophase (Pl. XXII, Figs. 16, 17; Pl. XXIII, Fig. 40). Later still the tiny nucleus is homogeneous and grey, except for the small nucleolus (Pl. XXII, Fig. 18).

#### DISCUSSION.

The general appearance of the resting nucleus is very similar to that described by Hartmann (13) for *Chlorogonium*. The appearance of definite rounded bodies in the peripheral parts of the nucleus during the early prophase of mitosis, as figured by this author, is also seen in the *Ulvaceae*. Hartmann, however, although he mentions the appearance of threads like a spireme stage, does not figure anything comparable to the definite threads observed during the present investigation, whilst in his later work on *Eudorina* (14) the question of the occurrence of a definite spireme is left an open matter. Hartmann (13) describes the origin of one half of the spindle before the other from a centriole, which becomes visible on the nuclear membrane after the disappearance of the nucleolus. He describes the division of this centriole, one part of it migrating to the opposite end of the nucleus, giving rise to the second half of the spindle. In *Eudorina* he observed the darkly staining centriole in

the periphery of the nucleus even in the resting condition. Later, he was able to distinguish the same body in *Chlorogonium* also. This centriole divides in the early stages of mitosis, and is responsible for the origin of the spindle. In the present investigation it has already been stated that spindles were too rarely encountered for it to be possible to say anything at all about their origin, but it can be stated with certainty that on several occasions such a conspicuous darkly staining granule was observed in the nucleus after its initial swelling preparatory to mitosis (Pl. XXII, Fig. 22, *c*), and also that in at least two of the perfect spindles observed there seemed to be a definite granule at one pole (Pl. XXIII, Fig. 35). Thus, whilst direct evidence was not obtained, the probability is that the spindle is of the same nature and origin in the Ulvaceae as in *Chlorogonium* and *Eudorina*. Hartmann (13) states that in the metaphase the chromosomes seem to be united in pairs. Hartmann believes that this fact is responsible for the controversy existing as to the number of chromosomes in the Phytomonadineae (l. c., p. 23), and the present writer is inclined to think that it has also something to do with the difficulties she has experienced in the present work, and also with the fact that the three species examined did not seem to conform as regards chromosome number. An apparent union of the chromosomes in pairs is evident in many of the writer's drawings of the Ulvaceae (see Pl. XXIII, Figs. 42, 45), and, as has been mentioned previously, the cohesion of the chromosomes is often carried much farther, so that in the metaphase they form a solid mass. Taking into consideration the possibility of such a fusion of the chromosomes in pairs at the meta- and anaphases, it seems most likely that the true number of chromosomes in *Ulva* and *Monostroma latissimum* is really ten, the number which is suggested by the segmented form of the spireme. The groups of apparently five chromosomes passing to each pole in the anaphase probably represent ten fused together. Hartmann has already pointed out that this fusion of the chromosomes has nothing to do with reduction, these organisms being all haploid, reduction of the chromosomes taking place on the germination of the egg.

Hartmann describes for *Chlorogonium* the contraction of the chromosomes in the telophase to form what he considers a true karyosome, which later disintegrates into definite granules which become dispersed in the more peripheral regions of the nucleus, leaving behind a true nucleolus. Similar appearances to these telophase stages of Hartmann's for *Chlorogonium* were commonly seen in the Ulvaceae, yet, on account of the small size of the new nuclei, the present writer hesitates to say definitely that the smaller granules visible in the newly formed daughter nuclei were actually derived by fragmentation of the coalesced chromosomes, though it is, of course, possible that they really are formed in this manner.

As regards the Protococcales, Smith (25, 27) gives a little information on the method of nuclear division in *Characium* and *Tetraedron*

respectively, but unfortunately so few stages are figured that a comparison of these forms with the species under investigation is impossible. McAllister (17) figures for *Tetraspora* a long and convoluted spireme, which is much more comparable to that of the higher plants than any similar stage seen in *Ulva*.

In the Ulotrichales very little is known of the method of nuclear division. Oltmanns (19), although figuring somewhat diagrammatically two spindles with five chromosomes in *Colcochaete*, gives practically no information concerning the mitotic process. Allen (1) has given an account of nuclear phenomena during the germination of the zygote in *Coleochaete*, but he devotes more attention to the heterotypic mitosis, and as far as can be ascertained there is no striking similarity between the large and complicated nucleus of the form he studied and the tiny nucleus of the Ulvaceae. The chromosomes in the heterotypic mitosis were long and slender, and the nucleus in *Colcochaete* seems much more comparable with that of the higher plants than is apparently the case in the Ulvaceae. Haase (12) has given a short description of the process of cell-division in *Ulothrix subtilis*, but, apart from describing the nuclear figure as dumb-bell shaped, he does not dwell especially on the nuclear condition. Neuenstein (18) gives an account of mitosis in *Microspora amocna*, which embodies some new ideas. He describes the origin of the chromosomes in the peripheral region of the nucleus, and then the migration of these bodies to the nucleolus, around which they throng, so that they derive from that body a quantity of chromatin. He never observed a spireme to be formed. In accordance with the experience of the present writer, the spindle here was also very rarely observed, and the chromosomes divided on the equatorial plate. They were small rounded bodies. Even at this stage the remains of the nucleolus were visible. One of Neuenstein's figures shows the solid mass or bar of chromatin in the equatorial plate stage very similar to the appearance so frequent in *Ulva*, except for the presence in Neuenstein's figures of the remnants of the nucleolus, and the nuclear membrane still clearly visible. Neuenstein's description of the telophase is peculiar in that he portrays the daughter chromosomes crowding round the newly formed nucleolus, and giving up to it their chromatin material, after which they retreat and become broken up into the small chromatin granules of the network. The same worker made an attempt to investigate the nuclear condition of *Draparnaldia* and *Stigeoclonium*, but had much difficulty owing to the small size of the nucleus, and the impossibility of distinguishing between the nucleus and the pyrenoid. He was unable to give any information as to the division of the nucleus in these forms.

Neuenstein's interpretation of the behaviour of the chromatin at the telophase in *Microspora* is suggestive of Hartmann's description of the same stage in *Chlorogonium*. The former believes that the chromosomes aggregate



and give up their chromatin to the nucleolus, and then become dispersed in the peripheral region of the nucleus, whilst the latter describes the contraction of the chromosomes to form first of all a true karyosome, which later disintegrates, the chromatin being dispersed in the peripheral region of the nucleus, leaving behind a true nucleolus. It seems probable to the writer that these two accounts are simply the different interpretations of two human minds of the same process, and this indicates that the utmost care should be taken in interpreting isolated stages of mitosis, a process which must of necessity be studied in fixed material, and cannot be observed satisfactorily in the living alga.

#### *Cell-division.*

Vegetative cell-division in *Ulva lactuca* is peculiar in the form of the cleavage plane. A recently divided cell shows, as a rule, not a straight cleavage plane going right across the cell, but one with several undulations (Pl. XXII, Figs. 20-23). Careful focusing shows that the septum or cleavage plane is most undulating chiefly in the lower part of the cell, or, in other words, in the region of the nucleus, at the lower margin of the protoplast. Cleavage begins first of all at that end of the cell where the nuclei lie, between the daughter nuclei, extending gradually upwards to the upper part of the protoplast where the chloroplast and pyrenoid are located. Before there is any sign of cleavage in the upper part of the protoplast the lower half of the cell often shows conspicuous undulations in the cleavage plane (Pl. XXII, Fig. 22, *a, b*). Sections at right angles to the surface of the frond show that the undulations are not confined to one plane, but can be seen in the lateral view of the cell (Pl. XXII, Fig. 21). As cleavage becomes more and more complete, the cleavage plane becomes straightened out, and by the time the new cell-wall is laid down, the division between the daughter cells is quite straight. The writer does not remember ever having seen or heard of such irregular cleavage planes previously in a case of simple cell-division. D'Arcy Thompson (29) has explained the occurrence of S-shaped walls dividing certain long cells, and Smith (23) has described curved cleavage planes in the formation of autocolonies in *Tetrademus*, yet these examples do not show anything like the pronounced undulations seen in *Ulva*. The writer is quite at a loss to explain the prevalence of these irregular dividing planes. In the species of *Monostroma* examined, the cleavage planes were apparently quite normal.

#### FORMATION OF GAMETES AND ZOOGONIDIA.

Some reference has already been made to the formation of swarm-spores. Whether the reproductive cells are to be sexual or asexual their formation is very similar. Schiller has already studied the formation of

gametes in *Ulva*. In the present case only zoogonidia (4-ciliate) were observed in *Ulva*, and only gametes (2-ciliate) in *Monostroma latissimum*. *M. Gracile* was not observed to produce any kind of motile spores. In general the formation of gametes in *Monostroma* was found to conform very well with the account given by Schiller for *Ulva*.

In *Ulva* the fruiting thallus has been very beautifully figured by Thuret (30, Pl. II, Fig. 1), showing the fertile margins of the frond, colourless after the release of the swarm-spores. The writer has observed zoogonidia formation in *Ulva* to begin in groups, just a few rows of cells from the margin, extending inwards as time goes on from these points. During the gamete formation in *Monostroma latissimum*, practically the whole thallus may form motile spores, which, on their escape, leave nothing but the filmy remains of the cell-walls. In both species the swarm-spores escape by a pore, the two rows of cells in *Ulva* releasing their spores by somewhat projecting pores on both sides of the thallus, and in *Monostroma* the cells opening with pores always on the same side of the thallus. The division of the protoplast takes place at night between 11 p.m. and 4 a.m., and the swarm-spores are released in the early morning.

Cytologically the process is comparatively simple. The cells involved in swarm-spore formation increase considerably in size and their nuclei swell enormously (Pl. XXII, Fig. 2). Nuclear division and cleavage of the cytoplasm occur with great regularity, so that at each division uninucleate protoplasts result (Pl. XXII, Fig. 24; Pl. XXIII, Figs. 43, 44, 53-56). Only very rarely does nuclear division get ahead of cleavage, resulting in the formation of multinucleate protoplasts (Pl. XXIII, Fig. 49). In the later stages mitosis takes place in all the tiny protoplasts simultaneously, so that all the nuclei in a cell are in the same condition at any one time. This is often a most conspicuous feature (Pl. XXIII, Figs. 50, 51, 53-56).

The structure of the gametes and their conjugation has been described in *Ulva* by Schiller (21), and the gametes in *Monostroma* are similar to those of *Ulva*, both in their structure and in their physiological reactions. Schiller, however, does not seem to have found any difference in size of the gametes in connexion with their sexuality, so that in *Ulva* we have a real case of isogamy. In *Monostroma* a very slight difference in size of the gametes was discerned, the smaller gametes being presumably ♂ (Pl. XXIII, Fig. 57), and the larger ones ♀ (Pl. XXIII, Fig. 58). There is considerable variation in size of the ♂ and ♀ gametes, so that some of the larger ♂ gametes may be as large as the average ♀ gamete, or even larger; whilst some of the smaller ♀ gametes may be equal to or even smaller than the average ♂ gamete in size. On the whole, however, the ♀ gametes are slightly larger than the ♂ gametes. Moreover, ♂ gametes are produced only by a plant which is distinctly ♂, and similarly for the ♀ gametes. The *Monostroma* plant is thus dioecious and produces gametes of one sex only, which are incapable of conjugating between themselves. A similar case of sex

differentiation in an isogamous green alga was noticed many years ago by Berthold (2) in *Dasycladus claviformis*. Berthold, however, could detect no differences between the ♂ and ♀ plants of this alga, neither in their morphological structure nor in their corresponding gametes, yet there was a physiological<sup>1</sup> distinction of sex, since the gametes produced by any particular plant could not be made to conjugate amongst themselves. It would be very interesting to know how far dioecism occurs among the isogamous algae. It occurs frequently among the fungi, where + and – strains have been shown to occur since Blakeslee's historic work on the Mucorineae; also in the higher fungi, both the Ascomycetes and the Basidiomycetes. Bristol (4, p. 477) has made the statement that in *Chlorococcum* the gametes produced by a cell may fuse amongst themselves or with those produced by a different cell, and it is not supposed as a general rule that the isogamous green algae are possessed of any highly differentiated sexuality. The fact that in at least two such algae, *Dasycladus* and *Monostroma*, the individual plants are of a distinct sex, suggests that physiological sex differentiation is possibly more common in these lowly plants than has been previously supposed. It would be interesting to know how many of the isogamous Chlorophyceae are really monoecious.<sup>1</sup>

Schiller found that the gametes in *Ulva* are liberated and swarm in the early morning. An exactly similar release of gametes was observed in *Monostroma*. If a number of plants are separated in a vessel and left overnight it is probable that next morning there will be a green cloud of gametes in the part of the vessel best illuminated. The physiological experiments on light described by Schiller using *Ulva* gametes can be repeated successfully with *Monostroma* gametes.

Some interesting experiments were performed with the *Monostroma* gametes in connexion with their sexuality and reactions to light. The plants were rinsed after being collected and placed as soon as possible in sea-water, each plant in a separate vessel. On the next day or the succeeding two or three days the gametes were liberated and collected as described above around the lightest parts of the vessel. Using a sterile pipette for each transfer, to prevent the accidental mixing of the two sexes, samples of the gametes from two of the vessels were brought together in turn in

<sup>1</sup> The writer has recently noted two such cases amongst the Chlorophyceae. *Protosiphon botryoides* has been observed to produce isogametes which sometimes conjugate within the mother coenocyte amongst themselves without being released, producing tiny star-like zygospores. The segments of a single plant of *Hydrodictyon utriculatum* produce gametes which readily conjugate amongst themselves. Klebs (16) has also observed the monoecious nature of *Hydrodictyon*, and also of *Phyllobium*. Dodel (8) has recorded that the gametes from a single filament of *Ulothrix zonata* conjugate amongst themselves, whilst Hieronymus (15) has also observed the same thing in *Gnium*, *Chlorogonium*, and *Stephanosphaera*. Of other algae, *Vaucheria* is undoubtedly monoecious, whilst *Spirogyra* includes both monoecious and dioecious forms, those species showing lateral conjugation being monoecious, and those with scalariform conjugation dioecious.

a small solid watch-glass. It was possible to tell from macroscopic observation if the two plants under investigation were of the same or of different sex. This could be diagnosed in the following way: If the light is sufficiently strong, and the watch-glass containing the gametes (recently mixed from two plants) be placed in front of a window, the gametes will be seen to seek at once the lightest part of the vessel, forming a green streak or cloud. Within 10 minutes, however, if the gametes are of the opposite sex, and are therefore conjugating, the green cloud begins to recede and soon has sought the darkest part of the vessel and has taken up its position there. Microscopic examination shows that the receding cloud consists entirely of conjugated gametes. At the end of 30 minutes practically all the conjugated gametes have found the darkest part, which is now occupied by a green cloud, instead of the lightest region as before. If the dish is now carefully rotated, so that the green cloud is made to occupy again the lightest part of the vessel, the zygotes at once begin to recede, and in 15 minutes have again taken possession of the darkest region. Thus it seems that conjugation is accompanied by a change in the physiological reaction of the organism to light, for unless conjugation takes place the gametes remain positively phototactic, whereas conjugation rapidly produces a change to a negative phototactic response. This sensitiveness to light is usually attributed to the presence of an eye-spot or stigma, but although both gametes and zygozoospores perceive light through the agency of the stigma, the reactions of the two kinds of spores are exactly the opposite of one another. The biological significance of these reactions is, however, fairly clear. As has already been suggested by Schiller in connexion with *Ulva* gametes, the positive phototaxis of the gametes is undoubtedly useful in bringing them together in great numbers at the surface of rock-pools, so that they have more opportunity of coming into contact with each other. It is also undoubtedly a good thing for the zygote to be negatively phototactic, seeking out dark corners, possibly under stones, &c., where they can rest undisturbed during the long months which must elapse before they can germinate. The sensitiveness of the zygotes to strong light is very remarkable. The watch-glass in which some conjugated gametes had sought out the darkest region, remote from the window, was darkened by a box with one side removed in such a way that the part of the dish nearest the window was made darker than the part of the dish containing the gametes. Thus the part of the dish originally light and destitute of zygotes was now dark, whilst that part containing the gametes was not appreciably altered as regards light. Yet even so the zygotes again began to swim across to the darkest region of the dish, now nearest the window. A few zygotes remained behind, so that now there were two clouds of green zygotes. Microscopic examination showed that the zygotes remaining behind had already lost their cilia so that they were unable to respond to light. The

cilia are only retained for an hour after conjugation, after which time the zygote has no power of locomotion and begins to acquire a cell-wall.

#### GERMINATION OF THE ZYGOTES AND PARTHENOSPORES.

The germination of the zygote in the Ulvaceae is a somewhat controversial subject, owing to the fact that there is much diversity in the observations of previous investigators.

Reinke (20), working on *Monostroma bullosum*, Thur., observed conjugation of isogametes in this alga. The resulting zygote, after acquiring a cell-wall, gradually increases in size for seven or eight weeks, after which germination took place in some individuals, but in others germination did not take place for five months. In the germination of the large spherical zygote the contents become divided into eight cells, arranged round the periphery. Cell-division proceeds in such a way as to preserve the structure of a hollow sphere, which is the form of the young *Monostroma* plant.

Chodat (6), working on the same plant, many years later, observed the zygote to divide into two and then into four to form a simple filament which gave rise to a new *Monostroma* plant. Chodat implies that the zygospores germinate directly, and not after a period of rest as described by Reinke.

Schiller (21) found that conjugation in *Ulva* was followed by the immediate germination of the zygote. Gametes developing parthenogenetically behaved in exactly the same way, except that germination in their case was delayed two or three days.

Unfortunately, the development of the zygote and parthenospores was not carried very far in the present investigation, but sufficient observations were made to confirm the statements of Reinke rather than those of Chodat for *Monostroma* and of Schiller for *Ulva*. The gametes observed by the writer were liberated in April 1922. Zygotes resulting from the conjugation of the gametes, and also gametes of both sexes, which had not been permitted to come into contact with gametes of the opposite sex, were kept under observation until the end of June. At different periods also until this time, cover-glasses on which the gametes or zygotes had been allowed to settle were removed from the vessel and fixed for cytological examination. At the end of June the writer moved from the East Atlantic coast, and was unable to have any longer the facilities of a marine location. The spores were kept under observation until June 1923 but no germination was observed, although some of them were at that time still green and apparently living.

Zygotes, ♂ and ♀ parthenospores, all behaved in the same way during their early development. All three are first transformed into resting spores, and there is no appreciable difference in size of the resting cells resulting from the different spores. Almost immediately after conjugation and a short period of swarming in the conjugated and 4-ciliate condition (Pl. XXIII, Figs. 59, 60), the zygote rounded itself off, and having sought the darkest

possible corner, comes to rest (Pl. XXIII, Figs. 61, 62). On the following day both zygotes and such parthenospores as have come to rest are seen to have undergone a kind of germination out of the delicate membrane with which they had become invested, the contents, which have increased in size, passing out, leaving the tiny spore-case behind as a delicate vesicle (Pl. XXIII, Fig. 63). The zygote is thus an elongated mass with the colourless spore-wall adhering to one side. The pigment spot, or in the case of the zygote the two pigment spots, are still distinctly visible, although they disappear in the next day or two. The zygotes or parthenospores then swell out and increase enormously in size, becoming pear-shaped or oval and finally spherical in form. In eight days they are usually distinctly pyriform in shape, and the remains of the original spore-wall can often be distinguished (Pl. XXIII, Fig. 66). With the increase in size the resting spores appear to become densely green and full of starch. The contents of the final resting spore must be many hundreds of times greater than that of the original zygozoospore.

Cytological examination of the zygotes and parthenospores showed that in the case of the zygotes the nuclei of the gametes had not yet fused in a zygote three days old, in which two pyrenoids and two nuclei could readily be seen (Pl. XXIII, Fig. 65, *c.*). In zygotes thirteen days old the nuclei in all individuals had united (Pl. XXIII, Fig. 67). Complete fusion of the contents of the zygote therefore takes place at some time between the third and thirteenth day after conjugation. The pyrenoids of the two gametes evidently retain their individuality.

The zygote or parthenospore at the twentieth day is a rounded body with a stout cell-wall about  $20-30\mu$  in diameter. Male parthenospores kept separately under observation showed that some of these may occasionally greatly exceed the female parthenospores in growth. There is much variation in size of both zygotes and parthenospores (cf. Pl. XXIII, Figs. 68, 69). At the twenty-seventh day the spores have increased in size to about  $30-35\mu$  (Pl. XXIII, Fig. 70), and at about the thirty-eighth day they have attained their maximum size, about  $50\mu$  (Pl. XXIII, Fig. 71). At this stage they are densely green and full of starch, and may contain several pyrenoids. After this time no further changes were observed.

#### SUMMARY.

The cell-structure of *Ulva lactuca*, *Monostroma Grevillei*, var. *VahlII*, and *M. latissimum* is essentially the same. In *Monostroma*, however, the cells forming the stalk and holdfast remain uninucleate and do not become multinucleate as in *Ulva*.

In *Ulva*, the fronds of which are two layers in thickness, the chloroplasts with the pyrenoids lie on the external walls of the cells, and the nuclei on the inner walls in the middle of the thallus. In *Monostroma*,

which consists of a single layer of cells, the chloroplasts all lie normally on one surface of the frond and all the nuclei on the opposite side.

In vegetative cell-division the pyrenoid does not usually disappear entirely during mitosis. The pyreno-crystal may, however, lose some of its staining properties, probably because of the partial solution of its food reserves, or it may be broken by fissures, small particles being distributed in the peripheral cytoplasm of the cell. In the first case, one of the daughter-cells resulting from the division receives the feebly staining pyrenoid, whilst the second daughter-cell develops a pyrenoid *de novo*. In the second case, the remnant of the original pyrenoid serves as the pyrenoid of one daughter-cell, and in the other daughter-cell one or more of the fragments become reorganized to form pyrenoids. These two methods are not confined to particular species, and probably occur in combination sometimes within a single cell.

The nuclei are exceedingly small, but their division is essentially mitotic. A spireme, distinctly segmented, is a striking feature, and the number of chromosomes is probably ten. There is a decided tendency for the chromosomes to fuse, often into a solid mass, in the meta- and anaphases.

The cleavage plane dividing the daughter-cells in *Ulva* is at first undulating, but before the new cell-wall is laid down it has become straightened out.

Swarm-spores, either sexual or non-sexual, are formed by successive nuclear divisions and cleavage of the protoplast. In this process it seems that the pyrenoids behave essentially as in vegetative cell-division, and they may be re-formed after each cleavage.

In *M. latissimum* the plants are dioecious, gametes from any single plant failing to conjugate amongst themselves, and only producing zygotes when brought into contact with gametes of another plant of the opposite sex. There was much variation in size in both ♂ and ♀ gametes, but on the whole a slight difference in size according to the sex could be distinguished.

Gametes of both sexes may develop parthenogenetically in exactly the same way as zygotes. The spores all increase tremendously in size for about forty days, and form a large thick-walled resting cyst about 50  $\mu$  in diameter, which does not germinate for several months. The development of the new *Monostroma* plant from the cyst was not observed.

#### ADDENDUM.

Since this work has gone to the press the writer has seen a paper by J. Schiller—Beobachtungen über die Entwicklung des roten Augenfleckes bei *Ulva lactuca* ('Oest. botan. Zeitschr.,' lxxii, p. 236, 1923). Schiller describes and figures, in the development of the gametes in *Ulva*, a body similar to that illustrated in Pl. XXII, Figs. 1, 19, s. of the present work. According

to Schiller, this body undergoes successive divisions as the protoplast of the cell divides to form gametes, so that each gamete finally receives a part of it in the form of an eye-spot. It is very probable that the body described by Schiller is the same as that observed by the writer. Schiller also supports the present writer's observation that during gamete formation the pyrenoid is visible after each division of the protoplast.

## EXPLANATION OF PLATES XXII AND XXIII.

Illustrating Dr. Nellie Carter's paper on the Cytology and Biology of the Ulvaceae.

### PLATE XXII.

*n.*, nucleus; *s.*, see text.

Figs. 1-25, *Ulva lactuca*.

Fig. 1. Portion of thallus in surface view, just preparatory to cell-division.  $\times 1,354$ .

Fig. 2. Part of thallus preparing for zoogonidia formation.  $\times 1,354$ .

Figs. 3-18. Vegetative mitosis.  $\times 1,600$ . Fig. 3. Early prophase. Figs. 4, 5. Showing segmented spireme. Fig. 6. Indication of splitting of spireme. Fig. 7. The equatorial plate stage from the side. Figs. 8, 9. Polar view of metaphase. Figs. 10-12. Anaphase. Figs. 13-18. Telophases.

Fig. 19. Part of thallus in vertical section.  $\times 1,600$ .

Fig. 20. A dividing vegetative cell.  $\times 1,354$ . *a*, focused on the upper surface; *b*, at the level of the nuclei.

Fig. 21. A similar cell in vertical section at right angles to cleavage plane.  $\times 1,354$ .

Fig. 22. Portion of thallus in surface view.  $\times 1,354$ . *a*, cell in surface focus; *b*, the same cell at a lower focus.

Fig. 23. A dividing vegetative cell.  $\times 1,354$ . *a*, in surface; *b*, at the level of the nuclei; *c*, focusing on the lower wall.

Fig. 24. Two cells with zoogonidia nearly fully formed.  $\times 1,354$ . Each protoplast has both pyrenoid and nucleus visible.

Fig. 25. Showing fragmentation of the pyrenoids.  $\times 1,600$ .

Figs. 26, 27, *Monostroma Grevillei*, var. *VahlII*.

Fig. 26. A few cells from lower part of thallus.  $\times 500$ .

Fig. 27. Portion of thallus.  $\times 1,354$ . Cells in various stages of division.

### PLATE XXIII.

Figs. 28-71, *Monostroma latissimum*.

Figs. 28-40. Vegetative mitosis. Fig. 28. Portion of thallus.  $\times 1,354$ . Nuclei in early prophase. Fig. 29. Prophase.  $\times 1,354$ . Figs. 30-3. Spireme stages. *n*, nucleolus. Fig. 31.  $\times 1,600$ . Figs. 32, 33.  $\times 1,354$ . Fig. 34. Indication of doubling of spireme.  $\times 1,354$ . Figs. 35, 36. Metaphase.  $\times 1,354$ . Figs. 37-9. Anaphase. Figs. 37, 38.  $\times 1,354$ . Fig. 39.  $\times 1,600$ . Fig. 40. Late telophase.  $\times 1,354$ .

Figs. 41-54. Reproductive mitosis in gamete formation. Fig. 41. Cell in prophase.  $\times 1,600$ . Fig. 42. Metaphase of first mitosis, showing two pairs of chromosomes and three apparently fused



pairs.  $\times 1,354$ . Fig. 43. Telophase of first mitosis.  $\times 1,354$ . Fig. 44. Prophase of second mitosis.  $\times 1,354$ . Fig. 45. Metaphase of second mitosis.  $\times 1,600$ . Figs. 46-7. Anaphase of second mitosis.  $\times 1,354$ . Figs. 48, 49. Final stage of second mitosis.  $\times 1,354$ . Fig. 50. Metaphase of third mitosis.  $\times 1,600$ . Fig. 51. Anaphase of third mitosis.  $\times 1,600$ . Fig. 52. Completion of third mitosis, pyrenoids reformed, nuclei invisible.  $\times 1,354$ . Figs. 53-6. Later stages in the formation of gametes.  $\times 1,600$ .

Fig. 57. Male gametes from a  $\sigma^7$  plant.  $\times 1,600$ . *st.*, eye-spot; *py.*, pyrenoid.

Fig. 58. Female gametes from a  $\phi$  plant.  $\times 1,600$ .

Figs. 59, 60. Conjugation of gametes.  $\times 1,600$ .

Figs. 61, 62. More mature zygotes.  $\times 1,600$ .

Fig. 63. Examples from a mixed culture of gametes and parthenospores one day old, living material.  $\times 1,600$ . *p.*, parthenospore; *z.*, zygospore; *sp.*, original spore membrane.

Fig. 64. The same on the second day.  $\times 1,600$ . (Stained.) *n.*, nucleus.

Fig. 65. The same on the third day.  $\times 1,600$ . (Stained.)

Fig. 66. The same on the eighth day,  $\times 1,600$ , from living material.

Fig. 67. The same on the thirteenth day.  $\times 1,600$ . (Note single nucleus in all the spores.)

Fig. 68. Male parthenospores, nineteen days old.  $\times 840$ . Living material.

Fig. 69. Female parthenospores, seventeen days old.  $\times 840$ .

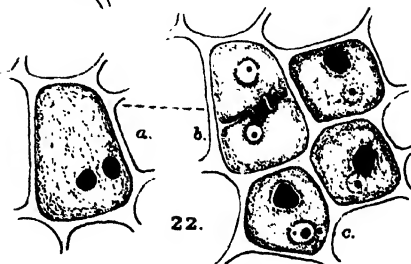
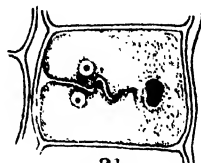
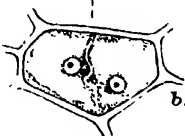
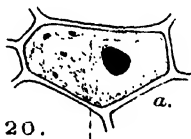
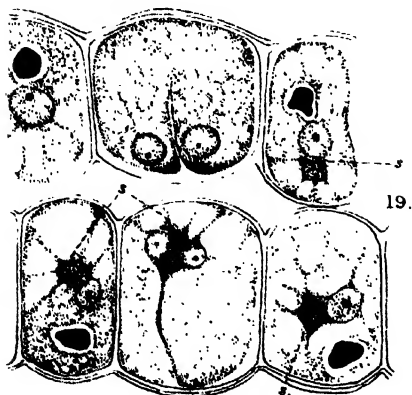
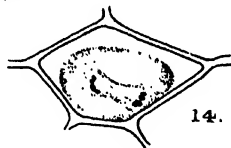
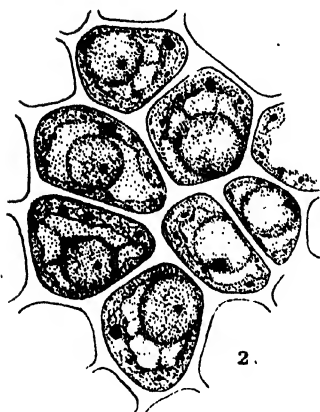
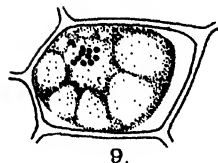
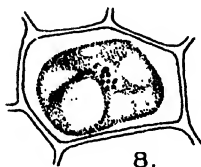
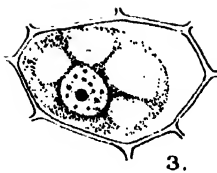
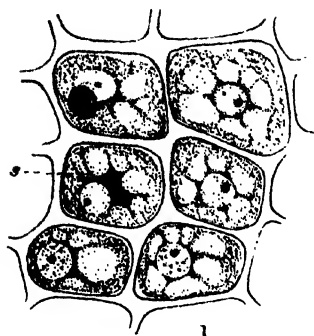
Fig. 70. From a culture of mixed zygotes and parthenospores, twenty-seven days old.  $\times 550$ .

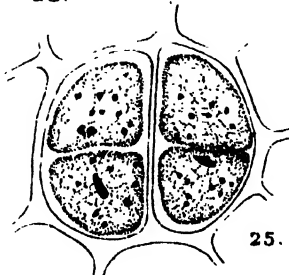
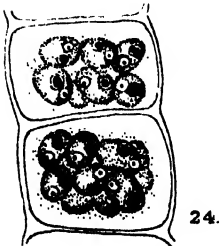
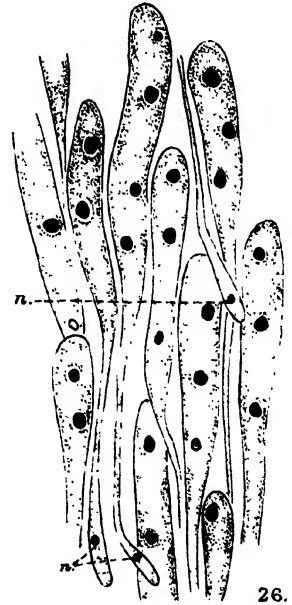
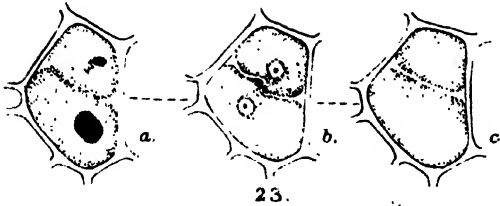
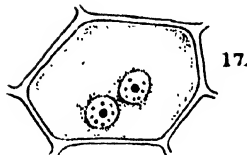
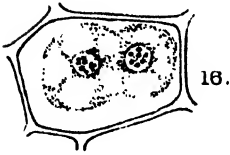
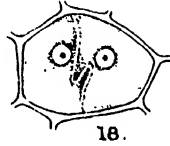
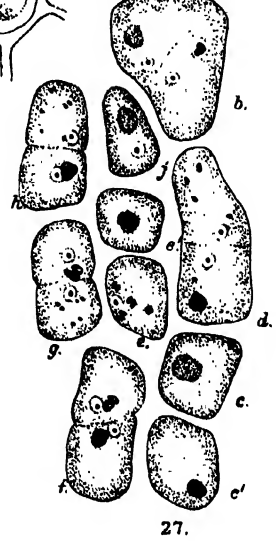
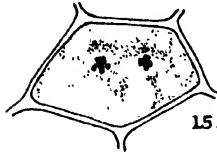
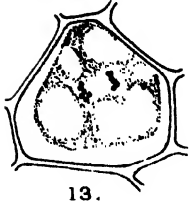
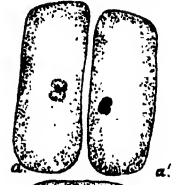
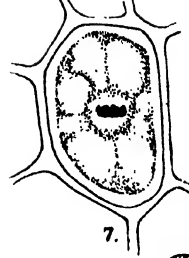
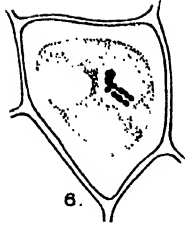
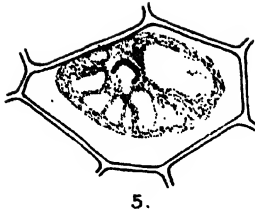
Fig. 71. The same at the age of thirty-eight days.  $\times 550$ .

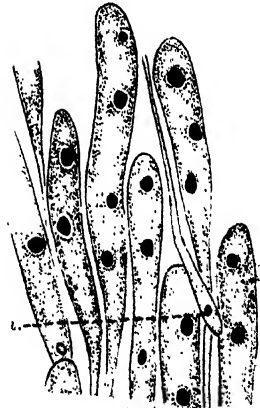
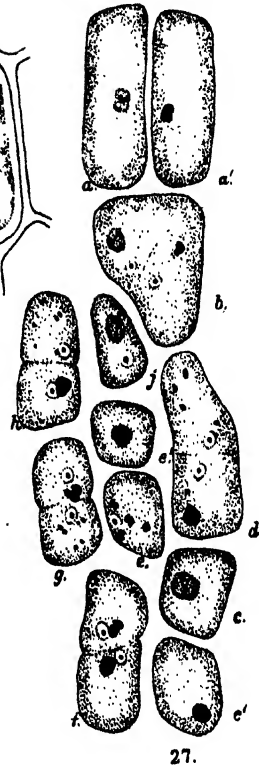
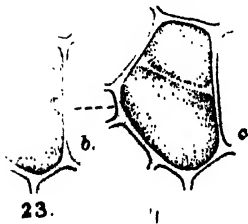
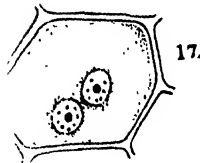
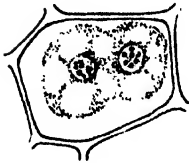
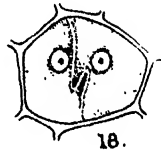
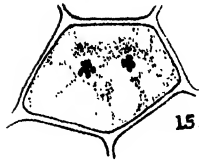
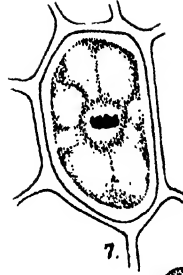
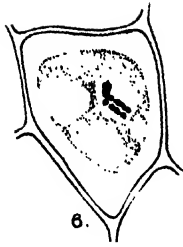
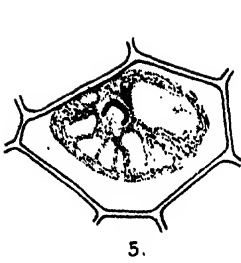
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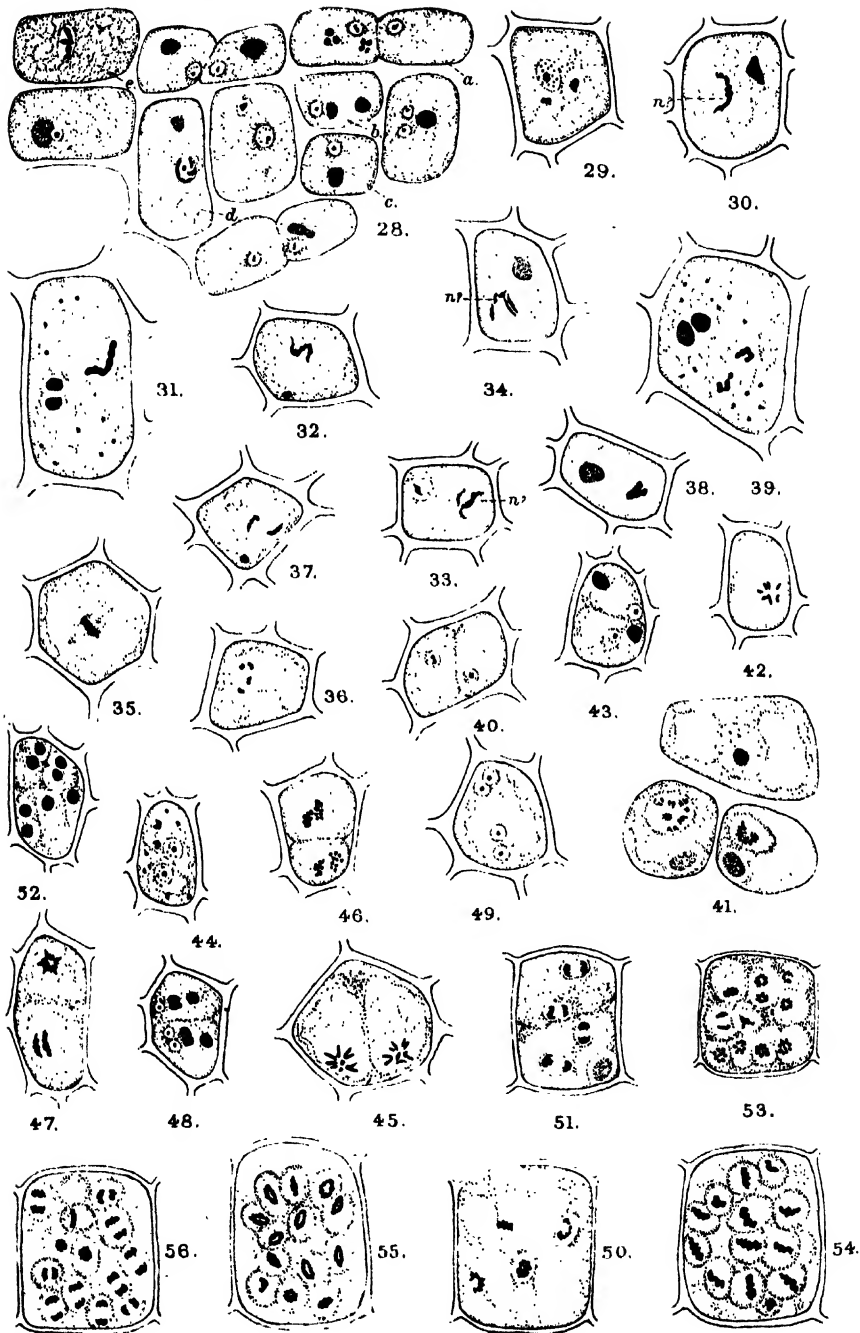
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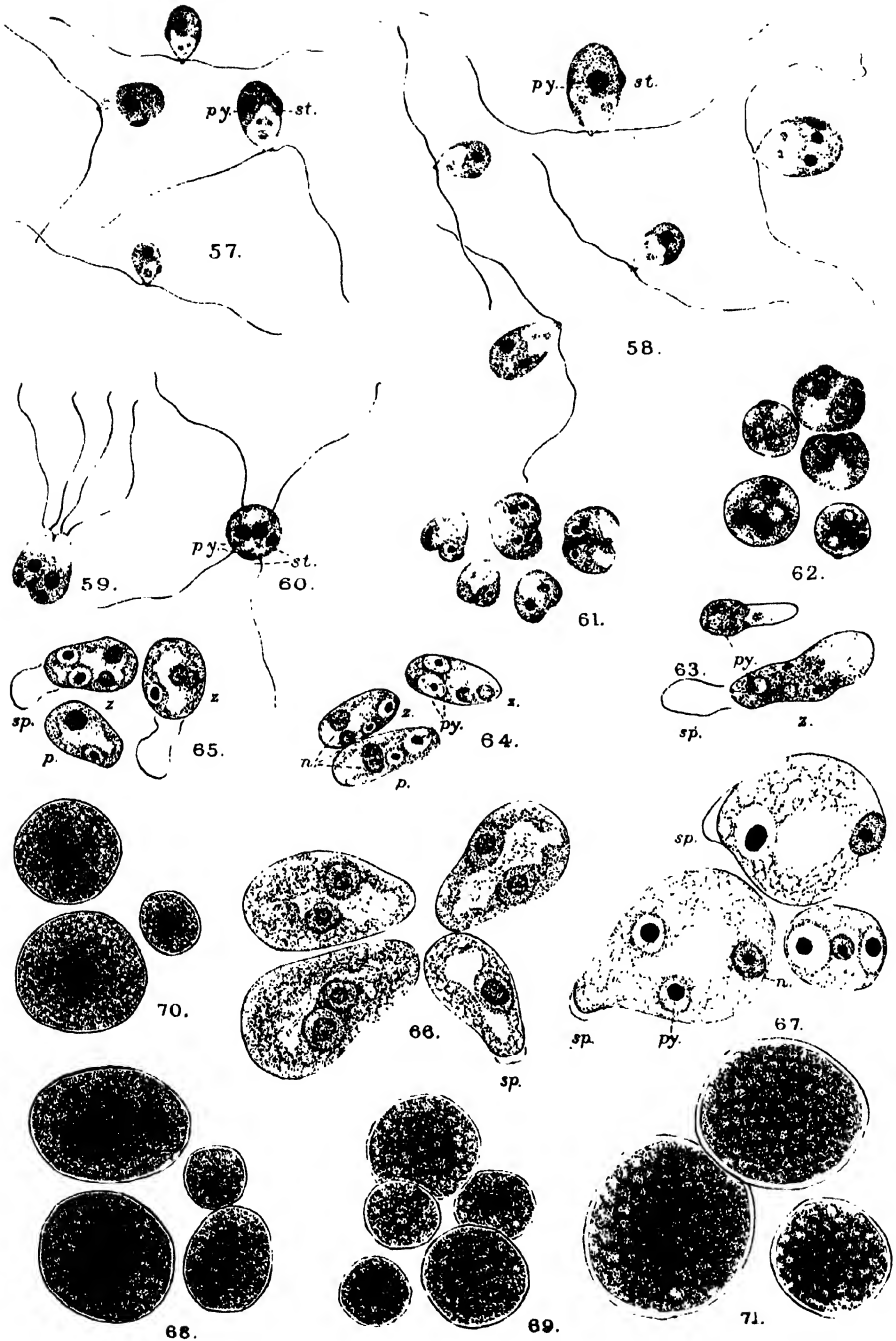








N.C. del.







# Studies in the Cytology of the Anacrogynae.

## I. Antherozoids.

BY

AMOS M. SHOWALTER.

With Plate XXIV and three Figures in the Text.

THIS paper and two which are to follow are the result of studies pursued during two years of residence in Belgium as an Exchange Fellow of the C. R. B. Educational Foundation. All of the experimental work, except the attempts to hybridize *Fossombronina* with *Sphacrocarpus* and with *Funaria*, was conducted while in Belgium, and nearly all the permanent microscopical preparations were made in the Institut Botanique of Brussels. The critical examination of the microscopical preparations and the writing of the papers are being done under the tenure of a National Research Fellowship in the Biological Sciences.

During my residence abroad Professors V. Grégoire of Louvain and J. Massart of Brussels accorded me the facilities of their laboratories and many personal courtesies. The Jardin Botanique de l'État at Brussels provided facilities for extensive cultures. Professor Ch. Killian of Strasbourg and Mr. D. A. Jones of Harlech, Wales, each collected and sent me living plants from his respective region, and Professor H. van den Broeck of Antwerp made numerous identifications of species. Many other European and English botanists also made contributions of lesser importance. Professor and Madame A. Rutot offered me the generous hospitality of their home and so contributed much to the success of my work.

Acknowledgements are due also to the University of Wisconsin for laboratory and library facilities, and to Professor C. E. Allen, who has given many helpful suggestions and criticisms during the preparation of the papers.

### VARIETIES OF *RICCARDIA PINGUIS*.

It is well known that the form and size of the thallus of many hepatics vary greatly with the conditions under which they grow. The differences between plants of the same species collected at different stations are sometimes regarded as resulting from direct responses to the climatic and

ecological conditions under which the plants have grown. Mansion (1905) says of *Riccardia pinguis* (L.) S. F. Gray: 'Espèce des plus variables, mais presque toujours facile à ramener au type, à cause, précisément, de sa fragilité à l'état humide. Les nombreuses formes de l'*A. (R.) pinguis* sont intéressantes, parce qu'elles montrent bien l'adaptation directe de l'espèce aux conditions changeantes du milieu dans lequel elle végète.'

Plants for the study of fertilization in this species were secured from near Stambach (Alsace), Brussels, and Winterslag (province of Limbourg), and cultivated in the Bryophytes greenhouse of the Jardin Botanique at Brussels. The plants from these different sources were noticeably different when collected, but no genetic significance was at first attached to these differences. The differences persisted, however, and were as distinct after the plants had been cultivated under similar conditions for two seasons as they were in the original collections. Attempts to intercross them seem to show that there are also genetic differences. Representative plants with sporogones from these cultures were sent to Professor A. W. Evans, who finds also differences in the sporogones. The characters of the different varieties or races have not been analysed systematically, and I shall give here only such characters as were noted in my work.

For convenience I shall designate the different forms as types A, B, and C. The plants collected near Stambach are designated provisionally as *R. pinguis*, type A, those collected near Brussels as *R. pinguis*, type B, and those collected near Winterslag as *R. pinguis*, type C.

The original collection of plants of type A was made near Stambach, early in February 1923, by Professor Ch. Killian and sent to me at Brussels. They had grown on a black soil mixed with decaying wood and other vegetable matter, but were found to grow as well on the non-humous calcareous clay of Brussels as on leaf mould. The thallus is dark green, rather sparingly branched, 5–8 mm. wide, and often attaining a length of 8 cm. Cross-sections of the thallus (except near the young growing end) show fungal hyphae in many of the cells of the ventral region, and in some of the rhizoids. When fixed in the chromic-osmic-acetic acid solution (formula in succeeding paper) and washed, the thallus remains somewhat darkened, although not nearly so much as thalli of *Pellia* killed in the same solution. The plants of this type have grown more luxuriantly in culture, and have seemed less susceptible to disease than those of types B and C. The sexual branches, usually in groups of three, are less numerous in type A than in the other two types, and the archegonia are only partially covered by papillate scales. The antherozoid, exclusive of its cilia, measures 73–81  $\mu$  in length.

The plants of type B were obtained at two stations near Brussels, one station in the Forêt de Soignes, near l'Abbaye de Rouge Cloître, and the other in the prairie at the edge of the forest near Trois Couleurs. At both

these stations the plants grow on a calcareous clay soil which is very poor in humus. The thallus is only slightly smaller than that of type A, branches more freely, is slightly less dark in colour, usually free from fungal hyphae (when healthy), and when killed in the chromic-osmic-acetic acid solution and washed, is darkened only in the meristematic regions, the rest of the thallus being semi-translucent. The sexual branches, usually in groups of three or five, are more numerous than in the plants of type A, and the archegonia are covered by an abundant growth of papillate scales. The antherozoid has approximately the same length as that of type A.

The original plants of type C were collected April 30, 1923, at the edge of a pond near Winterslag, in the sand-dune region of eastern Belgium. They were small and appeared so unpromising that only a few were collected and placed in culture. These were for the most part male, and grew very little the first season, but produced a large number of sexual branches and a few sporogones. The thallus is small, 2-4 mm. wide, branches freely, and seldom attains a length of more than 3 cm. No fixations were made and no examination for fungal hyphae while the plants were in healthy condition. The plants of this type began producing antheridia about two months later than those of the other two types (which had been in culture during part or all of the winter) and continued to yield antherozoids until the end of October. The culture was later attacked by fungal parasites, and I succeeded in saving only a few plants of each sex. The sexual branches, usually borne singly, are numerous but very short, the papillate scales on the archegonial branches few and small. The antherozoid is markedly shorter than that of either of the other two types, measuring 53-64  $\mu$  in length.

Female plants of type A inseminated with antherozoids of type C fruited abundantly. The reciprocal cross was not made for lack of cultures of type C female plants. Female plants of type A inseminated with antherozoids of type B produced no embryos large enough to be seen with the hand-lens; this experiment was repeated several times. Cytological studies (of which an account will appear later) show that the antherozoids enter the eggs and that development begins, but that the gametic nuclei do not fuse normally. Female plants of type B inseminated with antherozoids of type A produced sporophytes, most of which aborted before reaching maturity.<sup>1</sup> Female plants of type B inseminated with antherozoids of type C produced no sporophytes, but no microscopical examination was made to determine whether or not there were eggs ready for fertilization at the time of insemination.

<sup>1</sup> This insemination was made June 30, 1924. Two months later, at the time of my return to America, about a score of young sporophytes were developing in the culture. Dr. A. M. Wolfson cared for this culture and reported that all except three of the sporophytes aborted although the plants seemed to be otherwise in healthy condition.

THE ANTHEROZOIDS OF *RICCARDIA PINGUIS*.

The season during which antherozoids of this species were readily obtainable in my cultures at the Jardin Botanique began in May and ended early in September. After a few initial experiments antherozoids could be obtained when desired for fertilization experiments and for study.

The antheridia are borne on the dorsal sides of short lateral branches of the thallus. These branches occur in clusters of three or five or, in type C, singly. The antheridia are easily recognizable with the unaided eye because of their large size and light colour, and because they are only partially embedded in the tissues of the antheridial branches. The male plants were kept relatively free from surface moisture by watering only from below and not to excess. Being kept in a humid atmosphere, very little watering was necessary. Male plants bearing mature antheridia and kept for a few days free from excessive moisture rarely failed to discharge antherozoids when lifted off the soil and put into water. After a few minutes the plants were replaced in the culture, and in many cases were used again successfully the second or third day afterwards.

A small culture of plants of type C grown from stock collected in the sand-dune region of eastern Belgium served as the source of the antherozoids used in nearly all the experiments recorded in this paper and for nearly all my permanent preparations of antherozoids. The plants of this form were found to be especially favourable for these experiments because they produce few rhizoids and do not attach themselves firmly to the soil. Thus they are easily removed from the culture and replaced without serious disturbance. Antheridial branches and antheridia were very numerous in this culture.

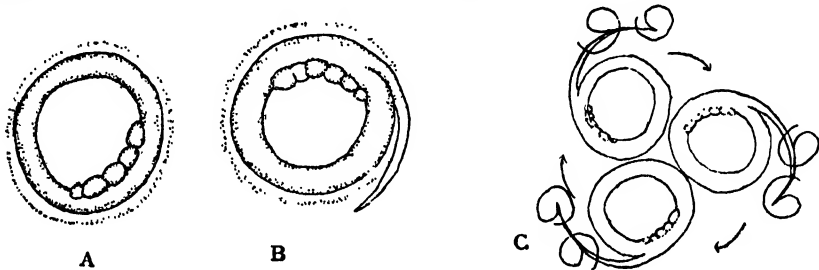
Male plants of type B brought in from the prairie south-east of Brussels after a few days of rainless weather and placed in water also gave antherozoids in abundance.

The discharge of the antherozoids as seen under the binocular dissecting microscope shows a whitish mass emerging from the antheridium and forming a small irregular column, suggesting in appearance a miniature column of smoke. In most cases the efflux is too slow to be perceptible except by very critical attention. The column of whitish material usually rises towards the free surface of the water, becoming larger in diameter and less definite in outline. The column may attain a length of a millimetre or more, but more frequently the substance of which it is composed is dispersed before it attains half that length. Antheridia, presumably over-ripe, frequently discharge a whitish mass of similar appearance which contains no functional antherozoids. In some cases, especially in the case of over-ripe antheridia, the whitish mass discharged shows a tendency to sink rather than to rise.

The discharge of the contents of a considerable number of antheridia

was observed. The size of the column of matter discharged, the speed of its emergence, and the time of its dispersal vary considerably, but in no case have I found any approach to an 'explosive discharge' such as occurs in *Conocephalum* (Thuret, 1856, Cavers, 1903 *a*, King, 1903), *Asterella* (Peirce, 1902), and other Marchantiaceae (Cavers, 1903 *b*). The last-named author observed that in *Riccia glauca* and in several members of the Anacrogynae the antherozoids are 'discharged quietly'.

When observed under the 15 mm. objective, the whitish mass exuded from the antheridium appears to be composed of small particles which separate by sudden jerky movements as though they were repelling one another, or as though the water entering between were breaking them apart. These particles rise to the surface of the water and float usually for a few



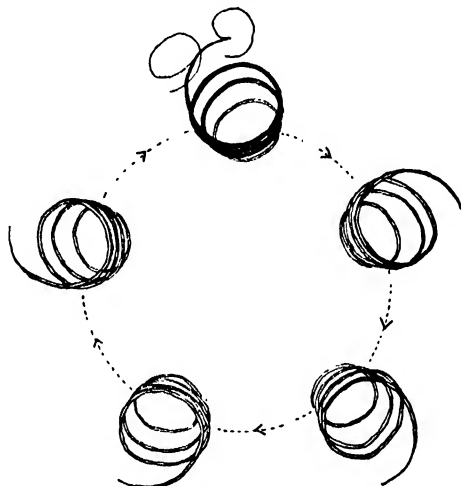
TEXT-FIG. 1. *Riccardia pinguis*. A. Freshly discharged antherozoid entirely enclosed in its gelatinous envelope. B. Anterior end projecting from gelatinous envelope. C. Diagram of antherozoid in three successive positions during its gyratory movements at the surface of the water.

minutes before any active movement is discernible. Floating at the surface they have the appearance of small perforated discs, in each of which an antherozoid is enclosed. Each antherozoid, including its cilia, is coiled in the outer region of the disc, and the central region is so translucent as to appear empty. The 4 mm. objective shows a series of refractive globules lying against the inner surface of the coiled antherozoid and extending from one-fourth to one-half the distance around the coil (Text-fig. 1, A). The gelatinous substance enclosing the antherozoid and composing the rest of the disc is scarcely visible when floating at the surface of the water, and escaped my observation until after it had been found in stained preparations. It is a definite envelope and was described and figured by Schacht for *Pellia epiphylla* as long ago as 1864.

The first noticeable activity of the antherozoid begins while it is still enclosed in the gelatinous envelope and floating at the surface of the water. This activity is first perceptible as a gentle rotation or spinning of the whole disc about its vertical axis and is not at first accompanied by any perceptible displacement with reference to the point of observation. The turning of the disc is perceptible (under the 4-mm. objective) because of the change of position of the refractive globules. It may be inferred that a part, at least, of the anterior cilium which is not visible has emerged from

the gelatinous envelope and has begun to strike the water. In the case of vigorous antherozoids this spinning or rotation may begin within a few minutes, rarely less than one minute or more than fifteen minutes, after the antherozoids reach the surface of the water. This rotation continues, at very uniform speed, ordinarily for from one to several minutes. The direction of this rotation when viewed from above is usually clockwise. Perhaps five per cent. of the cases observed showed a counter-clockwise rotation.

Very soon after the beginning of the rotation or spinning, sometimes almost simultaneously with it, the anterior end of the antherozoid is seen to



TEXT-FIG. 2. Diagram of five successive positions of the antherozoid in late phase of its gyratory movements.

extend outward from the coil (Text-fig. 1, B). The antherozoid now rotates about an axis slightly displaced, in the direction opposite the free end, from the centre of the coil or disc. It may be inferred that the direction of the pull of the cilia is not tangential to the coil but obliquely outward. As more of the body of the antherozoid separates from the coil the axis of rotation is further displaced, and soon the whole antherozoid is rotating or swinging about a point outside its own body. Text-fig. 1, C, represents diagrammatically three successive positions of an antherozoid during this movement. The time required to make a complete turn varies considerably with different antherozoids, but for the same individual is fairly uniform. One turn per second seems to be a fairly approximate average for vigorous antherozoids.

The magnitude of the circular orbit through which the antherozoid travels increases continuously, and in extreme cases may have a diameter several times that of the coil of the antherozoid. Text-fig. 2 represents five successive positions of the antherozoid just before the termination of this

movement. It will be noted that the anterior ciliate end of the antherozoid remains turned away from the axis about which the antherozoid rotates.

As the antherozoid proceeds along this circular orbit it becomes apparent that it is no longer coiled in the manner of a watch-spring, but that it is acquiring the form of an inverted conical spiral. The downward distension of the inner coils is visible from above (15 mm. objective) in consequence of the sharp inclination of the long axis of the spirally coiled antherozoid to the plane of the circular orbit (Text-fig. 2). This inclination of the axis of the antherozoid to the plane of its orbit results from the fact that the apex of the spiral (actually below) remains nearer to the axis of rotation than does the opposite end where the cilia are borne. This movement is terminated by an abrupt rotation through an angle of  $90^\circ$  with reference to the surface of the water (hence also with reference to the plane of the circular orbit), after which the antherozoid appears as a corkscrew-shaped body which swims freely and apparently aimlessly about in the water, free from the gelatinous envelope. It seems reasonable to infer that the movements thus far described are the means by which the antherozoid frees itself from the gelatinous envelope, and that the process may be prolonged or brief according to the tenacity with which the envelope adheres to the antherozoid. In a very few instances I have seen the antherozoid swimming horizontally in a fairly direct course with the envelope adhering to its posterior extremity and being dragged through the water.

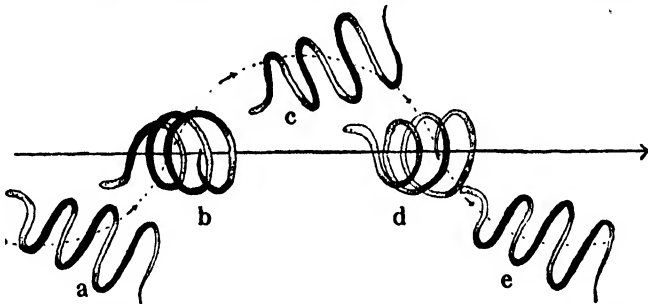
The gelatinous envelope is visible, during the movements just described, only as a small mass of transparent substance containing a few minute granules. Its outer limits are not sharply defined, and on the living unstained antherozoid it is only with difficulty recognizable as a definite structure. In permanent preparations of antherozoids fixed during these movements and stained differentially, the antherozoid is found in various positions relative to the envelope (Pl. XXIV, Figs. 4-7). In only one instance have I seen an antherozoid rotating inside the envelope without causing the latter to turn.

The refractive globules seen adhering to the inner surface of the coil before the antherozoid begins its gyratory movements remain visible during these movements, and are especially noticeable in the posterior portion of the body of the free-swimming antherozoid (Pl. XXIV, Figs. 2, 3).

The body of the free-swimming antherozoid has the form of a rod coiled in a spiral of from three to four turns. The diameter of the coils decreases from the anterior to the posterior end. The length of the enclosed cone is commonly about one and one-half times its largest diameter. The cilia, two in number and borne near the anterior end, are not visible in the actively swimming antherozoid because of the rapidity of their movements.

When swimming freely in water the antherozoid exhibits three distinct

movements, due presumably to its shape and to the oblique position of the cilia. It rotates on its own axis, swings about the axis of the direction of its progress, and moves forward. In the diagram (Text-fig. 3) the arrow represents the general direction of displacement through the water. The five spiral figures represent five successive positions of the antherozoid as it moves along its spiral course. This course, represented by the dotted line, is constantly at a left-forward oblique angle to the direction of progress. In the first position (*a*) the antherozoid is in the same plane as the axis of progress (arrow), but moving upward and forward. In the second position (*b*) it has revolved through an arc of  $90^\circ$  with reference to the axis of progress and is now above the latter. It has also rotated about the axis of its own body through an angle of  $90^\circ$  with reference to the point of observation. In the third position (*c*) it has revolved and rotated through



TEXT-FIG. 3. Diagrammatic representation of the antherozoid in five successive positions while free-swimming.

another  $90^\circ$  and is again in the plane of the axis of progress, but is now moving forward and downward. In the fourth position (*d*) it lies below the axis of progress; and in the fifth position (*e*) it has completed one revolution about the axis of its progress, has completely rotated about the axis of its own body, and has progressed along its course. The anterior end of the antherozoid remains constantly the part which deviates farthest from the axis of progress and points in the direction of its movement. Thus in position *b* this end is turned obliquely upward (towards the point of observation), and in position *d* it is turned obliquely downward.

The speed of these movements seems slow in comparison to that of the movements of a fern antherozoid (*Pteris aquilina*). The difference is due probably to the smaller number of cilia and to the relatively large size of the body. An attempt to measure the rate of movement may be described as follows: A drop of water containing a few actively swimming antherozoids is placed on a clean slide (without cover-glass). The drop spreads out to a thinness which allows free movements only in the horizontal plane, but does not interfere with the natural revolution of the antherozoid about the axis of its progress. The slide is placed on the stage of the microscope under the low-power objective (Zeiss 15 mm.). When an antherozoid is



seen to be swimming actively and proceeding in a fairly direct course, the slide is shifted so as to bring this antherozoid to the centre of the field of the microscope and the time is recorded. If the antherozoid continues to proceed in the same direction to the edge of the field, the time is again recorded. If it changes its course before reaching the edge of the field, the slide is again shifted so as to give it a fresh start at the centre. The readings thus obtained give intervals of 12 to 45 seconds, most frequently about 15 to 20 seconds. The stage micrometer shows this distance to be 0.6 mm. At this rate, about 2 mm. per minute, the antherozoid would be able to traverse a considerable distance in the few hours of its activity, but it rarely proceeds in the same direction for any long interval of time. An experiment described below will illustrate this point.

The direction of the spiral turns of the body of the antherozoid and also of its revolutions about the axis of its progress when swimming freely is that of a left-hand screw. This is very difficult to determine by observing either the swimming antherozoid or those lying inactive in the water. Free-swimming antherozoids and some which had come to rest on the slide were presented to two research workers in the laboratory with a request that they determine the direction of these turns. One of these men reported that the direction was that of a right-hand screw, and the other reported that some antherozoids were coiled in the one sense and that others were coiled in the inverse sense.

To determine definitely this point, the antherozoids were mounted in a solution of agar. A few drops of the warm agar solution (concentration about 2 per cent.) were poured on a warm slide and spread out so as to form a thin layer. A drop of water containing actively swimming antherozoids was placed on the layer of liquid agar solution and a cover-glass was added. The antherozoids continued to swim in this medium for several minutes, but as the solution congealed their movement retarded and ceased. The pulling of the cilia at the anterior end caused more or less distension of the coiled body, but many antherozoids retained nearly the same form exhibited when swimming in water (Pl. XXIV, Figs. 1-3). These preparations offer a means of observing critically the form of the antherozoid under high-power dry objectives, but are not sufficiently firm to permit a satisfactory use of the immersion objectives. Figs. 1-3 are camera lucida drawings of fresh antherozoids mounted in agar solution, but the cilia are here represented only diagrammatically.

About a hundred antherozoids examined in this way showed conclusively the form of a left-hand screw and none were found to show conclusively the opposite form. Guignard (1889) figures the antherozoids of *Pellia* and those of *Sphagnum* as though some were coiled in the one sense and others in the opposite sense, but he does not discuss this feature. Shaw (1898) finds that in *Onoclea* the spiral turns of the body of the anthero-

zoid are in the direction of a left-hand screw, and Webber (1897) reports that in the antherozoid of *Zamia* the helicoid band which bears the cilia is coiled in the same direction.

The time required for a discharged antherozoid to become free swimming varies greatly with different antherozoids from the same antheridium and still more with antherozoids from different antheridia. In several experiments the first observation of a free-swimming antherozoid was recorded two minutes after the male plants were placed in water. Most of the antherozoids liberated in these same experiments were not swimming freely until 10 to 15 minutes after their discharge from the antheridia. In other experiments very few of the antherozoids discharged showed even the rotating movement before 20 minutes and a very small percentage of them attained the free-swimming stage, although a considerable number of them were still rotating at the surface of the water at the end of two hours. In these latter cases the antherozoids were probably too feeble to free themselves entirely from the gelatinous envelopes.

Although much study has been devoted to these problems I have not been able to determine satisfactorily what force causes the freshly discharged antherozoids (enclosed in their gelatinous envelopes) to rise to the surface of the water or what part of the antherozoid is uppermost when floating at the surface. The rise of the antherozoids at once suggests a phenomenon of gravitation and leads one to assume that some part of the antherozoid or of the gelatinous envelope is less dense than water. The conspicuous refractive globules in the posterior end of the antherozoid were at first thought possibly to be buoyant, but the fact that this end of the antherozoid is below rather than above during the gyratory movements seems to negative this possibility.

In a number of experiments, thalli bearing ripe antheridia were immersed in water for about one second and placed dorsal side downward on slides which had been cleaned only by rubbing with a dry towel. A few drops of water were added by dipping the tip of a finger in water and touching the thallus. This brought the discharging antheridia nearer to the glass surface than to the free surface of the water, and the antherozoids discharged in these experiments descended and spread out to form a layer against the glass instead of rising. This suggests the probability of a phenomenon of surface tension, but my observations do not justify any final conclusion upon this question.

In several experiments a considerable number of antherozoids when emerging from the gelatinous envelope passed beyond the surface film of the water. These antherozoids were not coiled spirally but showed a serpentine form and glided about rapidly on the surface of the water. The same antherozoid could not be followed for a long period, but none of those under observation succeeded in piercing the surface film of the water. This

is evidence for the assumption that the surface of the antherozoid resists being wetted, but I have not succeeded in determining whether or not the same is true of the surface of the gelatinous envelope.

The movements of the antherozoid seem to be due entirely to the action of the cilia, the body being dragged about passively. The form of the body is readily altered by external physical forces, but I have been unable to detect any change in its form which might aid in locomotion. In the agar solution the pulling of the cilia at the anterior end distends the coils and in extreme cases draws the body into a linear form. When a drop of water containing swimming antherozoids is placed on a chemically clean slide, the surface tension of the water causes the bodies of the antherozoids near the edge of the drop to uncoil in various forms and often to become linear.

In several experiments antherozoids that had floated at the surface of water in a watch crystal and had begun to swim freely were left until their movements ceased. At the end of four hours after their discharge from the antheridia the antherozoids were observed to be accumulating at the bottom. Some of these at the bottom exhibited a few weak movements of the cilia, but it could not be determined whether their accumulation at the bottom was due to gravity or to their haphazard movements bringing them in contact with the glass to which they adhered. When all movement had ceased some antherozoids remained suspended in the water at various depths; a few were seen at the surface, but they were most numerous at the bottom.

At temperatures between 20° and 25° C., six hours seems to be about the limit of time for which any of the antherozoids are able to swim. Steil (1923) has noted that the antherozoids of this species remained motile for more than thirty minutes after they had been discharged from the antheridia. Thuret (1856) observed that the antherozoids of *Conocephalum* remained active for two days when the temperature was 'peu élevée'.

As noted above, the free-swimming antherozoids of *Riccardia* do not proceed in the same direction for any great distance. In one experiment a thallus bearing antheridia was dipped in water and placed dorsal surface downward on a slide which had been cleaned only by rubbing with a dry towel. A few drops of water were added from above until the water was about one millimetre in depth and formed a little pool about one centimetre long and half as wide. In a few minutes a mass of antherozoids in their envelopes was seen spreading out against the glass near one end of the pool. The thallus was removed without noticeable disturbance of the discharged mass of antherozoids. In a few minutes the antherozoids appeared as a seething mass, and at the end of ten minutes nearly all were swimming freely. These antherozoids swam as vigorously as any I have observed, but spread out very slowly to other parts of the pool. At the end of an hour after their discharge from the antheridium they were still crowded and jostling one

another where they were discharged, while near the other end of the pool, at a distance of less than a centimetre, only a few at a time were visible in the field of the 15 mm. objective.

The internal structure of the antherozoid can be studied to better advantage in stained preparations. A drop of water containing living antherozooids is placed on a clean slide and inverted for a few minutes over a solution of osmic acid. The water is then allowed to evaporate, and then the slide may be passed into the staining solution. A few minutes in a very dilute solution of gentian violet, followed by rinsing in water, drying, and the addition of balsam and a cover-glass, give preparations which show the general form of the antherozoid and some degree of differentiation of its parts. Giemsa's solution or safranin may be used, but both are less satisfactory than gentian violet. Heidenhain's iron-alum-haematoxylin has proved more successful in differentiating the parts of the antherozoid. After being mordanted in iron-alum, the slide is left in a 2 per cent. solution of haematoxylin for from four to twenty-four hours. Fifteen seconds to one minute in 2 per cent. solution of iron-alum is sufficient to differentiate the stain, one minute being about the limit if the cilia are to remain visible. Counter-staining with Bismarck brown is useful for some purposes, especially for showing the gelatinous envelope. Safranin and gentian violet were tried without success, as were also a number of other combination stains.

The general form of the antherozoid, that of a coiled rod-shaped body with two cilia at the anterior end, the posterior portion of the body being cytoplasmic, has long been known. Recently Steil (1923) has noted the unequal length of the two cilia and their insertion at two different points in the body. He noted also that 'a darker staining portion at the point of attachment of each cilium can always be observed when the antherozoid is not overstained'. In favourably differentiated haematoxylin preparations an oblong body more darkly stained than the nuclear rod which constitutes most of the body is recognizable at the base of each cilium (Pl. XXIV, Figs. 4-9). The anterior one of these two bodies appears to be smaller than the posterior one and is more apt to be destained by the differentiating solution. The body at the base of the posterior cilium occupies an oblique position with reference to the long axis of the body of the antherozoid and causes a slight hump in the surface (Pl. XXIV, Figs. 4-7). Each cilium seems to arise from the posterior end of its basal body, but I have not been able to trace the insertion as well as might be desired.

The major part of the body of the antherozoid is composed of a long, optically homogeneous rod pointed at either end and commonly regarded as the nucleus (Steil, 1923). This rod appears homogeneous, but its change in thickness accompanying its entrance into the egg leads me to suspect that its cortex is non-nuclear.<sup>1</sup> The anterior limit of the nuclear rod is not

<sup>1</sup> Fig. 35, of the succeeding paper of this series.

clearly defined in my preparations, but seems to be slightly anterior to the basal body of the posterior cilium. Its posterior end tapers to a sharp point (see also Steil, 1923), and in haematoxylin preparations is sharply differentiated from the cytoplasmic posterior portion of the antherozoid (Pl. XXIV, Figs. 8–11).

The cytoplasmic posterior portion of the body of the antherozoid varies greatly in form and composition (Pl. XXIV, Figs. 8–11, 13–14). The refractive globules so conspicuous in the living antherozoid are now seen to be vacuoles. The granular substance between the vacuoles, especially in the median portion of the cytoplasmic region, often stains more darkly than does the nuclear rod (Pl. XXIV, Figs. 8, 9, 13, 14). This cytoplasmic portion is readily stained with Bismarck brown.

The actual thickness of the nuclear rod is difficult to measure accurately, since its apparent thickness varies slightly with the intensity of the stain. The thickness in the median portion is about  $0.75\mu$ . The length of the entire body (exclusive of cilia) varies from  $53$  to  $64\mu$ , averaging about  $58\mu$ . The anterior cilium measures  $23$  to  $30\mu$  in length, averaging about  $27\mu$ ; the posterior cilium  $26$  to  $34\mu$ , averaging about  $30\mu$ . These measurements are for the antherozoids of *Riccardia pinguis*, type C.

The antherozoids of *R. pinguis*, type A, are not perceptibly different in thickness from those of type C, but are noticeably longer. The measurements are: for the body  $73$  to  $81\mu$ , average about  $77\mu$ ; anterior cilium  $28$  to  $34\mu$ , average about  $31.7\mu$ ; posterior cilium  $31$  to  $40\mu$ , average about  $37\mu$ .

Only a few antherozoids from the plants of type B were measured. These measurements all fall within the range of variation of the corresponding measurements given for type A, except those of the length of the posterior cilium, which are greater by a few microns.

I have found one antherozoid (from a plant of type C) which bears three cilia (Pl. XXIV, Fig. 12). These arise at three different points in the body of the antherozoid, and each cilium measures  $26\mu$  in length. The basal bodies do not show up well in this instance, and I am unable to say whether two or three of them are present.

For purposes of comparison a few observations were made on the antherozoids of a number of other hepatics. These observations are briefly noted.

#### *RICCARDIA MULTIFIDA*, (L.) S. F. GRAY.

The antherozoids of this species have been examined only superficially. The freshly discharged antherozoids are enclosed each in a gelatinous envelope similar to that described for *R. pinguis*, float at the surface of the water, and exhibit the same gyratory movements as do those of *R. pinguis*. The free-swimming antherozoid has essentially the same form and swims in the same manner. It is somewhat less easy to detect because of the thin-

ness of its body and the spiral is slightly more drawn out (see also Steil, 1928). Only a few permanent preparations were made, using the haematoxylin stain. The cilia are borne as in *R. pinguis*, but the basal bodies are somewhat less conspicuous. The body of the antherozoid measures about  $0.45\mu$  in thickness and about  $56\mu$  in length; the anterior cilium about  $25\mu$ ; the posterior cilium  $28\mu$  (Pl. XXIV, Figs. 15, 16).

*FOSSOMBRONIA ANGULOSA*, (DICKS.) RADDI.

The antherozoids of this plant are extremely difficult to observe when actively swimming, except with the use of the dark-field condenser. The spirally coiled body is decidedly more distended than are those of the antherozoids of the two species of *Riccardia* already described. In stained (haematoxylin) preparations the basal bodies stand out conspicuously in consequence of their large size relative to the thickness of the body of the antherozoid (Pl. XXIV, Figs. 17, 18). The cilia are borne at different points in the anterior part of the body. The posterior cytoplasmic 'vesicle' is very inconstant in form and commonly contains one or more darkly staining clumps. The body measures about  $0.2\mu$  in thickness and about  $47\mu$  in length; the anterior cilium is about  $27\mu$  long; the posterior cilium, about  $30\mu$ .

*PELLIA FABBRONIANA*, RADDI; *P. EPIPHYLLA*, (L.) CORDA;  
AND AN UNIDENTIFIED FORM OF *PELLIA*.

The discharge of the antherozoids in these three plants was observed to be similar to the process described for *Riccardia pinguis*. The freshly discharged antherozoids float at the surface of the water and exhibit the same gyratory movements, and the free-swimming antherozoids have the same general form as do those of *Riccardia pinguis*. The anterior part of the body is somewhat more slender than in the last-named species, and the basal bodies of the cilia are less prominent. In all three forms the posterior cytoplasmic 'vesicle' is very variable in form and size (Pl. XXIV, Figs. 19-24). The body of the antherozoid of *P. Fabbroniana* (Pl. XXIV, Figs. 19, 20) measures about  $0.5\mu$  in thickness and about  $60\mu$  in length; its anterior cilium is about  $35\mu$  long; the posterior cilium about  $38\mu$ . In *P. epiphylla* (Pl. XXIV, Figs. 21, 22) these measurements are: for the body about  $0.6\mu$  in thickness and  $60-80\mu$  (average about  $70\mu$ ) in length; for the anterior cilium about  $29\mu$  in length; for the posterior cilium about  $32\mu$ .

The unidentified form of *Pellia* was found (a single thallus) in a culture of *Riccardia pinguis* started with plants from the first collection made after my arrival in Belgium. This one plant proliferated freely and was propagated, but no archegones and of course no sporogones were produced. This plant produced hundreds of antheridia during a long season extending

from early spring to late summer. Female plants of *P. Fabbroniana* which were inseminated with antherozoids from this plant produced no sporophytes. A diligent search at the station where the original collection was made revealed no more plants of the same kind. In this form of *Pellia* the body of the antherozoid is about  $0.7\ \mu$  in thickness in the middle, decreasing gradually towards the anterior end (Pl. XXIV, Fig. 24). Its length ranges from  $85\ \mu$  to  $105\ \mu$  (average about  $95\ \mu$ ): its anterior cilium is about  $35\ \mu$  long, and its posterior cilium about  $38\ \mu$  (Pl. XXIV, Figs. 23, 24). Miyake (1899) says that the antherozoid of *Makinoa crispata*, (Steph.) Miyake, is larger than that of *Pellia*, but does not state with what species of *Pellia* his comparison was made, nor does he give any measurements. His figures, said to be drawn at a magnification of 900 diameters, show a length of 80 mm., thus indicating  $89\ \mu$  as the approximate length of antherozoids of *Makinoa*.<sup>1</sup>

*SPHAEROCARPOS DONNELLII*, AUST.

There are two points of interest in the antherozoids of this species when compared with those of *Riccardia*, *Pellia*, and *Fossombronia*, viz. the absence of the gelatinous envelope and a difference in staining reaction. In stained preparations of freshly discharged antherozoids the larger number lie coiled with the cilia wrapped about the body, but no indication of a gelatinous envelope is discernible (Pl. XXIV, Fig. 25). In the same preparations are found fragments of an amorphous granular matrix in which the coiled antherozoids are or have been embedded (Pl. XXIV, Fig. 32). Such fragments are also found in fresh mounts of living antherozoids. Before staining these fragments of the matrix have a whitish milky appearance, but the cavities within the matrix from which the antherozoids have escaped are distinctly visible.

In haematoxylin preparations the basal body (or bodies), the nucleus, and a small spherical body in the cytoplasm a few microns from the posterior end of the nucleus are stained darkly (Pl. XXIV, Figs. 25-7). I have not been able to determine whether both cilia arise from an elongate basal body or whether there are two basal bodies. The nucleus is sharply differentiated from the posterior cytoplasmic region, but its anterior limit is not clearly defined. Apparently it extends forward to the basal body, but this is not certain. The one dark-staining body in the cytoplasm of the posterior region seems to be fairly constant in size and to occupy a fairly definite position with reference to the posterior end of the nucleus, but other

<sup>1</sup> While this paper was in press I obtained near Eureka, California, antherozoids of *Pellia Neesiana* which are similar in form to those described above but are considerably larger. The dimensions are: median thickness of body  $0.9\ \mu$  to  $1\ \mu$ , length of body  $108\ \mu$  to  $144\ \mu$  (average of 23 measured,  $123\ \mu$ ), anterior cilium about  $47\ \mu$ , posterior cilium about  $51\ \mu$ . I am not sure about the presence of basal bodies in this species; if present they are very small.

similarly stained bodies may be present in various positions between it and the nucleus or in contact with the latter (Pl. XXIV, Figs. 26, 30, 31). The distal end of the cytoplasmic vesicle sometimes appears slightly more dense than the rest of the vesicle, but I am unable to discern any definite body in it. Rickett (1923) reports that the cytoplasmic vesicle is apparently soon lost; it seems always to be present in my preparations, which, however, were made of antherozoids freshly discharged from the antheridia.

A safranin-violet combination stain has been found satisfactory for the antherozoids of this species. The antherozoids are stained for 20 to 40 hours in a saturated solution of safranin and for 3 to 6 seconds in a very dilute (1 : 10,000) solution of crystal violet. The basal body (or bodies) and the spherical body (or bodies) in the cytoplasmic vesicle remain red, while the nucleus, cilia, and less dense part of the cytoplasm are light purple (Pl. XXIV, Figs. 29–31).

In these preparations one occasionally finds the two cilia with the basal body (or bodies) dissociated from the rest of the antherozoid (Pl. XXIV, Fig. 28).

Rickett (1923) has given the following measurements of the antherozoid of this species: thickness of body  $0.5\mu$ , average length of body  $18\mu$ , anterior cilium constantly  $44\mu$  in length, posterior cilium  $46.5$  to  $50.5\mu$ .

#### *CONOCEPHALUM CONICUM*, (L.) DUM.

In stained (haematoxylin) preparations of the forcibly discharged antherozoids caught on a slide and fixed without previous immersion in water, no trace was found of a gelatinous envelope or of a granular matrix. The antherozoids lie in pairs, partly coiled, and with the cilia wrapped about them (Pl. XXIV, Fig. 33). An antherozoid fixed after having begun to swim shows a short nuclear rod, two cilia borne near the anterior end of the rod, and a cytoplasmic vesicle at the posterior end (Pl. XXIV, Fig. 34). My preparations do not show conclusively whether or not the cilia originate in a basal body.

#### SUMMARY.

1. Three varieties or races of *Riccardia pinguis* are described, and designated respectively as types A, B, C.
2. The antherozoids of *Riccardia* and *Pellia* when discharged from the antheridia are enclosed each in a gelatinous envelope.
3. The movements of the antherozoid to free itself from the gelatinous envelope are described.
4. The antherozoid of *Riccardia pinguis*, type C, is shorter than those of the other two types of this species.



5. In *Riccardia*, *Pellia*, and *Fossombronia*, each antherozoid bears at different points in its body two cilia of unequal length. A dark staining body is found at the base of each cilium.

6. Measurements of the dimensions of these antherozoids are recorded.

7. The antherozoid of *Pellia Neesiana* is probably the largest as yet found in the Bryophyta.

8. The antherozoids of *Sphaerocarpos* when discharged from the antheridium are embedded in a gelatinous matrix, but no individual envelope is found.

9. In *Conocephalum* neither gelatinous envelope nor matrix is found.

*Bibliography at end of second paper.*

DEPARTMENT OF BOTANY,  
UNIVERSITY OF WISCONSIN,  
April, 1925.

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EXPLANATION OF PLATE XXIV.

Illustrating Dr. A. M. Showalter's paper on the Cytology of the Anacrogynae.

All figures were drawn with the aid of the camera lucida and reproduced without reduction. Figs. 1-3 were drawn from preparations of fresh antherozoids mounted in agar solution.  $\times 1,000$ . Figs. 4-34 were drawn from stained preparations.  $\times 2,100$ .

Figs. 1-3. Fresh antherozoids of *Riccardia pinguis*, type C, mounted in agar solution.

Figs. 4-7. Various stages of emergence of the antherozoid of *R. pinguis*, type C, from the gelatinous envelope; haematoxylin—Bismarck brown.

Figs. 8, 9. Antherozoids of *R. pinguis*, type C, uncoiled; haematoxylin.

Figs. 10, 11. Posterior ends of antherozoids of *R. pinguis*, type C; haematoxylin—Bismarck brown.

Fig. 12. Antherozoid of *R. pinguis*, type C, with three cilia; safranin.

Figs. 13, 14. Antherozoids of *R. pinguis*, type A; gentian violet.

Figs. 15, 16. Antherozoids of *R. multifida*; haematoxylin.

Figs. 17, 18. Antherozoids of *Fossombronia angulosa*; haematoxylin.

Figs. 19, 20. Antherozoids of *Pellia Fabbrianiana*; gentian violet.

Figs. 21, 22. Antherozoids of *Pellia epiphylla*; haematoxylin.

Figs. 23, 24. Antherozoids of *Pellia* (species unidentified); haematoxylin.

Figs. 25-7. Antherozoids of *Sphaerocarpos Donnellii*; haematoxylin.

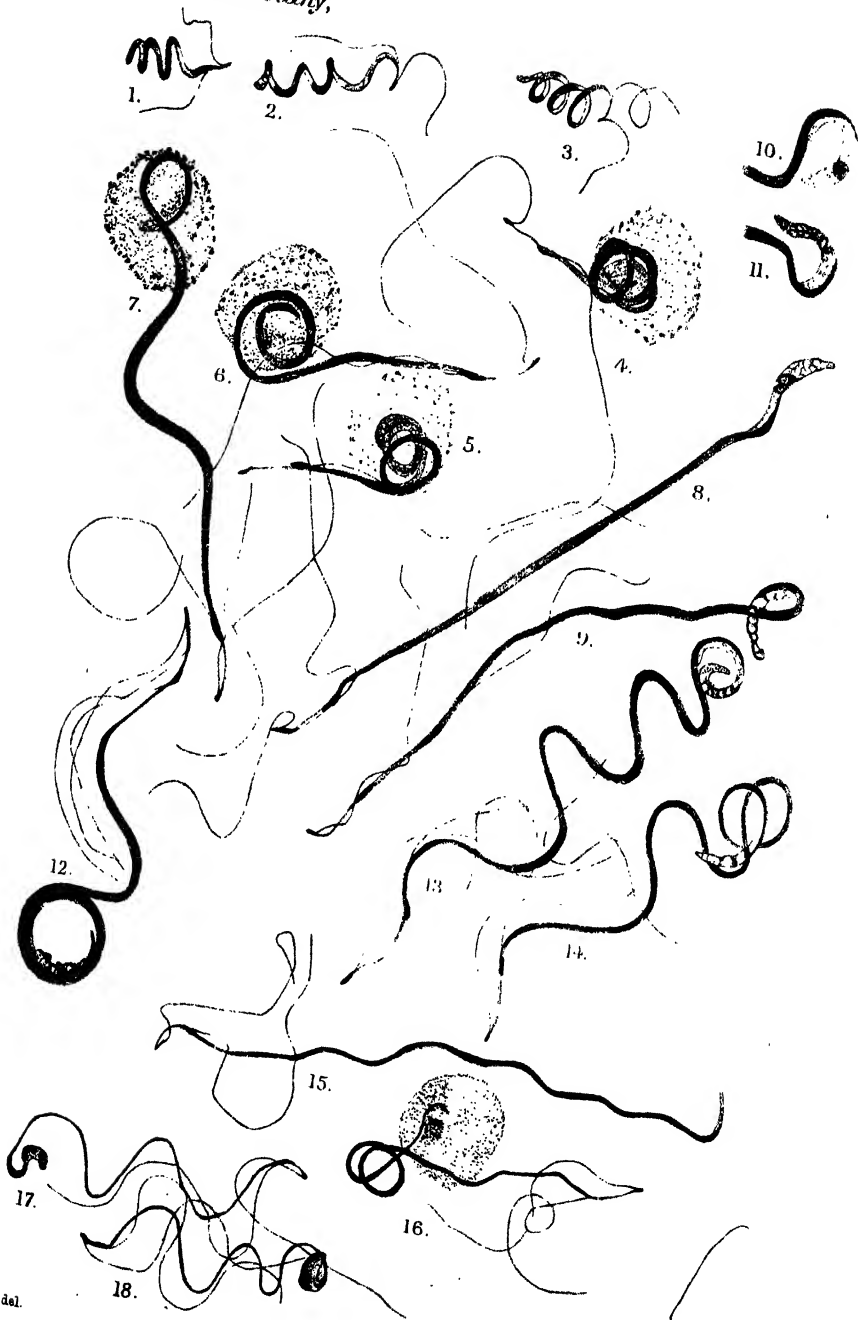
Fig. 28. Apical end and cilia of antherozoid of *S. Donnellii*; haematoxylin.

Figs. 29-31. Antherozoids of *S. Donnellii*; safranin-crystal violet.

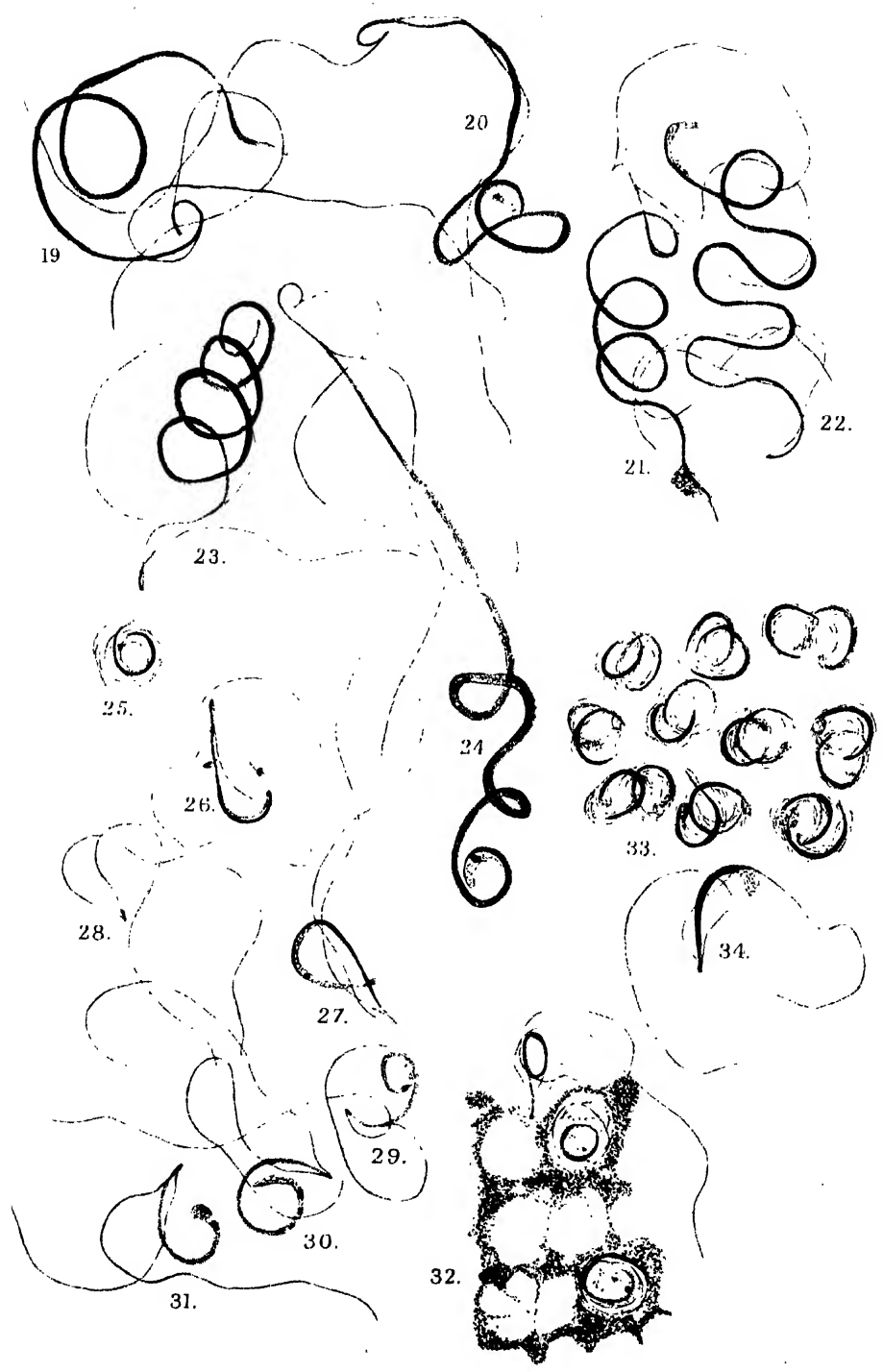
Fig. 32. Fragment of gelatinous matrix in which antherozoids of *S. Donnellii* are embedded when discharged from the antheridium; haematoxylin.

Fig. 33. Group of antherozoids of *Conocephalum conicum* immediately after their discharge from the antheridium; haematoxylin.

Fig. 34. Antherozoid of *C. conicum* after having attained the free-swimming condition.



A.M.S. del.





## NOTES.

**A NOTE ON THE OXIDATION AND REDUCING PROPERTIES OF HERMIDIN, THE CHROMOGEN OF MERCURIALIS.**—In a recent communication<sup>1</sup> dealing with the physiological significance of the chromogen of *Mercurialis*, it was pointed out that there exists in the plant a mechanism capable of reducing cyanohermidin to hermidin and possibly also chrysohermidin to cyanohermidin. It was also stated that the reduction of chrysohermidin could not be effected by sodium hydrosulphite. It has now been found that this statement is only true of samples of the pigment which have been kept for some time, since when freshly prepared it is readily reduced; even so short a time as two hours suffices to render the chrysohermidin incapable of reduction. That the secondary change undergone by the pigment is apparently not due to further oxidation was shown by dividing a freshly prepared solution into two parts, one of which was kept under nitrogen whilst the other was left exposed to the air; on testing after twelve hours both solutions were unattacked by sodium hydrosulphite, although the original solution, tested immediately after its preparation, had been readily reduced by this reagent.

In like manner the reduction of chrysohermidin to cyanohermidin by means of hermidin only takes place in freshly prepared solutions. To show this a solution of hermidin was run into the bottom of a nitrogen-filled tube containing a freshly prepared solution of chrysohermidin which had been rendered free from oxygen by alternately bubbling through nitrogen and exhausting. As the hermidin came in contact with the buttercup-yellow solution of chrysohermidin, an immediate blueing of the solution resulted. When repeated on a sample of the same chrysohermidin which had been kept for two hours, no blue colour was produced.

The unsuspected instability of chrysohermidin no doubt accounts for the varying results obtained in attempting to demonstrate the tissue reduction of chrysohermidin. It was pointed out in the communication referred to that on keeping a fully oxidized chrysohermidin solution in an atmosphere of nitrogen its colour gradually changed from yellow brown to olive green; this effect is more marked when the extract is prepared from young and vigorous shoots than when prepared from rhizomes, but in view of the fact that the reducing substance is present in limited amount and is, in any case, slow in its action, it is not to be expected that more than a trace of cyanohermidin will be produced, seeing that most of the chrysohermidin will have been transformed into the more stable substance before much reduction has been effected.

The circumstance that our colleague Mr. R. Cannan has submitted the oxidation-reduction potential of this system to a critical examination relieves us from proceeding farther with the study of the reversibility of the reaction.

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LONDON.  
March 1926.

<sup>1</sup> Haas and Hill, Ann. Bot., xxix, p. 861, 1925.

**A CELLULOSE-FILM TRANSFER METHOD IN THE STUDY OF FOSSIL PLANTS.**—In 1923 an account of a valuable new method of investigating fossil plants, preserved as flattened incrustations, was given in this Journal by Mr. J. Walton.<sup>1</sup> The essentials of this, which may be spoken of as Walton's Canada balsam transfer method, are briefly these: fastening the surface of rock with the fossil to a glass slide by means of a layer of heated Canada balsam of the right consistence; grinding away superfluous rock, without reaching or injuring the specimen; protecting the glass slide with paraffin wax while leaving the back surface of the rock exposed; placing the preparation thus protected in hydrofluoric acid to dissolve the mineral material of the rock. The result in suitable cases is to leave the organic material of the fossil firmly fastened to the Canada balsam on the slide and to expose to observation the side that had been turned towards the rock. Such preparations, after washing, can be kept dry and examined by reflected light, or be mounted in glycerine jelly under a cover-glass. They cannot be mounted in Canada balsam.

Mr. Walton's transfer method has for some time been employed in my laboratory in connexion with the study of fragmentary remains of Old Red Sandstone plants. Experience of certain of its inherent limitations led my laboratory assistant, Mr. E. Ashby, to work out another technique. This, though arising from Walton's method, is so distinct that it may be spoken of as Ashby's cellulose-film transfer method.

The method employed can be stated briefly by giving the main steps in order.

(a) Treat the surface to be transferred with a solution of cellulose acetate in amyl acetate. Other similar solutions can be used, e.g. the trade preparation 'necol', or a solution of celloidin.

(b) Allow the surface to dry thoroughly and, if necessary, repeat the treatment to obtain a sufficiently strong film.

(c) Grind away any superfluous rock, to lessen the mass to be dissolved.

(d) Place the specimen in hydrofluoric acid in a wax vessel until the cellulose film is freed and clear of mineral matter.

(e) Wash the transfer thoroughly in water.

(f) Dehydrate in 95 per cent. alcohol. (Absolute alcohol must not be used.)

(g) Clear in terpinol, oil of bergamot, &c. (Clove oil must not be used.)

(h) Mount in Canada balsam, applying slight pressure with a clip if necessary.

If a thin piece of rock has plant-remains on both surfaces, both can be treated, and two transfers obtained.

The fossils on the rock may be first macerated with Schulze's macerating fluid, washed thoroughly, and allowed to dry. The process above (a-h) then follows.

Ashby's cellulose-film transfer method gets rid of the troublesome details of technique of Walton's Canada balsam transfer method. There are never air bubbles in the preparation, and the film is not only transparent but level. But perhaps the most important limitation that is completely removed concerns permanent mounting. Since the cellulose film can be cleared and mounted flat in balsam, there is not only increased transparency but the preparation can be examined under high

<sup>1</sup> Ann. Bot., vol. xxxvii, pp. 379-91.

powers. This was usually impossible in the case of the irregular surface of Canada balsam covered with glycerine jelly.

The value and convenience of the new method have been thoroughly tested in work that is in progress, and it seems desirable to make it known to others engaged in similar investigations. While this account appears over my name I wish to make it quite clear that the conception, working out, and testing of the method have been entirely the work of my colleague, Mr. Ashby.

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# Studies in the Cytology of the Anacrogynae.

## II. Fertilization in *Riccardia pinguis*.<sup>1</sup>

BY

AMOS M. SHOWALTER.

With Plates XXV-XXVII and four Figures in the Text.

THE present study of fertilization in *Riccardia pinguis*, (L.) S. F. Gray, began with a few fragmentary observations made in the course of other cytological investigations in this species. These fragmentary observations have been published (Showalter, 1923 *a*) with slight alterations by the editor, who is responsible for the sentence containing the phrase '6 archégones fécondées'.

The account of fertilization in *Sphaerocarpos Donellii* by Rickett (1923) is the only adequate study as yet published of fertilization in a Bryophyte. Rickett summarizes all the various fragmentary observations of stages in the fertilization of Bryophytes which have appeared in the literature.

### MATERIAL AND METHODS.

The plants used in this study were grown in the Bryophyte greenhouse of the Jardin Botanique de l'État, Brussels, from stock collected by Professor Ch. Killian in February, 1923, near Stambach. They are, therefore, of the variety provisionally designated as *R. pinguis*, Type A, in the previous paper of this series. These plants grew well in culture and produced sporophytes in considerable abundance.

For fertilization studies plants of the two sexes were grown in separate cultures. Male plants of cultures kept in a fairly humid atmosphere, and watered only from below and only sufficiently to keep them turgid, yielded antherozoids in abundance when removed from the soil and placed in a dish of water. It was assumed that the conditions under which antherozoids are produced in, and liberated from, the male plants would be favourable also for

<sup>1</sup> The substance of this and of the succeeding paper was communicated verbally to the Botanical Society of America in session at Washington, D.C., December 30, 1924.

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the production of archegonia with eggs ready for fertilization. This assumption seems to have been justified by the number of fertilizations obtained.

Antherozoids for insemination were obtained by placing male plants for a few minutes in a Petri dish of water. The water was examined under the microscope, and, when the antherozoids were seen to be swimming freely, was poured over the female plants, which had been watered abundantly 15 to 30 minutes previously. This method of insemination gave relatively few fertilizations, due probably to the inability of the antherozoids to reach the archegonia. The method was consequently modified, the cultures of female plants to be inseminated being submerged in a photographic tray of water, so that the soil and about two-thirds of the thalli were below the surface of the water. After 15 to 30 minutes, water with antherozoids was poured over the cultures of female plants, which were left submerged for from 5 to 10 minutes. This method resulted in the fertilization of practically all the eggs in condition to be fertilized.

For cytological study, fixations of the inseminated female plants were made at short intervals up to the time when embryos were in evidence. The fixative used is a modification of the Flemming medium chromic-osmic-acetic acid solution having the following composition:

Chromic acid 1 per cent. (solution in distilled water)	200 c.c.
Osmic acid 2 per cent.	12.5 c.c.
Glacial acetic acid	3 or 6 c.c.
Distilled water	215 c.c.

The greater part of the material used in this study was fixed in the solution containing the higher concentration of acetic acid, but the best fixations were obtained with the lower concentration. Benda's solution for mitochondria, when diluted with an equal volume of distilled water, also gives good fixation of *Riccardia pinguis*.

The plants were left in the fixing solution for two or three days, were then washed in running water, and dehydrated with alcohol, beginning at 5 per cent. After dehydration the alcohol was replaced by chloroform, beginning with 1 part chloroform to 5 parts alcohol. It was found necessary to infiltrate gradually with soft paraffin, avoiding heat, in order to obtain satisfactory sections.

Sections were cut at  $10\mu$  thickness and stained with the triple stain. Heidenhain's iron-alum-haematoxylin gave results useful for comparison.

#### OBSERVATIONS.

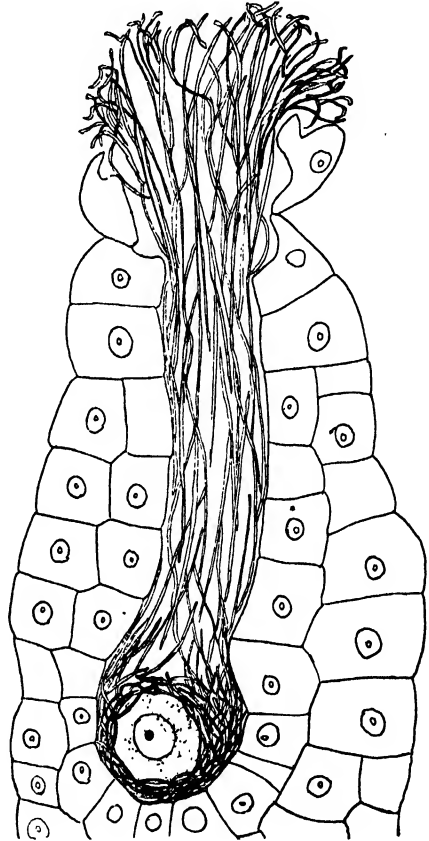
##### *Structure of the Egg.*

The egg lies at the bottom of the canal of the archegonium, which is short and wide. The neck canal is often almost as wide as the cavity of the venter (Text-fig. 1). In one case the fertilized egg lies in the neck near the

mouth of the archegonium instead of in the venter. The egg is usually spherical in shape, and its diameter (about  $20\mu$ ) is much less than that of the cavity of the archegonium (Pl. XXV, Figs. 35, 37). This failure of the egg to fill the ventral cavity is not due to a contraction during fixation, as is proven by the fact that the space about the egg is often completely filled with antherozoids (Text-fig. 1).

The spherical nucleus, about  $10\mu$  in diameter, lies near the centre of the egg. The chromatin is fairly evenly distributed throughout the nuclear cavity and appears as a reticulum in the fixed and stained material. The nucleole is large and usually spherical, but occasionally is elongate (Pl. XXV, Fig. 37). Very rarely there are two nucleoles (Pl. XXV, Fig. 39). The cytoplasm of the unfertilized egg is granular and quite dense, containing only very small vacuoles and a few denser bodies which are probably plastids. The cytoplasm stains darkly, especially with haematoxylin. The egg appears to be surrounded by a plasma membrane which undergoes no visible change either during or immediately after the entrance of the antherozoid. I find in the surface of the egg no 'receptive spot' or area which is more easily penetrable by the antherozoid than any other area. Rickett's (1923) careful study of *Sphaerocarpos* also revealed no indication of such a receptive spot. In some cases (especially of over-mature eggs) the side of the egg towards the mouth of the archegonium is flattened or even concave. A number of eggs of this form have been found in process of penetration by antherozoids, but in each case the antherozoid was penetrating in the convex region of the surface rather than in the flattened or concave region.

The substance of the disintegrated canal cells disappears so completely that scarcely any trace of stainable material remains around or above the egg. Rickett (1923) finds in *Sphaerocarpos* the neck of the archegonium



TEXT-FIG. 1. Longitudinal section of archegonium, 23 min. after a heavy insemination.  $\times 550$ .

filled with a mucilaginous material which interferes with the penetration of the fixing solutions and which is stained so darkly as to obscure from view the passage and penetration of the antherozoid. He thinks that the presence of this substance may have deterred cytologists from studying fertilization in the Bryophyta.

This study seems to indicate that the 'functional eggs' described in an earlier paper (Showalter, 1923 *b*) are in all probability fertilized eggs. My criticism of the figures of Miss Clapp and Florin was therefore unjustified.

Aberrant archegonia, some with more than one egg-like cell, have been described in many of the Bryophyta, and Florin (1922) has described a series of aberrant forms of archegonia in this species. Such aberrant archegonia are frequent in my preparations, and Florin's series might be extended, but I have found no case of fertilization in such aberrant archegonia.<sup>1</sup>

#### *Entrance of the Antherozoid into the Egg.*

The penetration of the antherozoid into the egg is a gradual process. Usually a number, sometimes a very large number, of antherozoids enter the archegonium. One antherozoid, probably the first which comes into contact with the egg, applies itself to the surface of the egg and is immediately reduced in thickness by 50 to 70 per cent. (Pl. XXV, Fig. 35). At the same time its staining reaction is altered. The antherozoid thus applied to the egg retains the haematoxylin stain more tenaciously than do other antherozoids in the same archegonium but not applied to the egg. With a properly balanced and differentiated triple stain the antherozoid thus applied (or, as is more probable, the nucleus of the antherozoid) is bright red in colour while the other antherozoids in the same venter are blue or purple. These supernumerary antherozoids in sections 10  $\mu$  thick are necessarily cut into relatively short pieces, and with the achromatic objectives the cut ends appear to be stained differentially—that is, the violet stain (or haematoxylin) appears to be more intense in the cortex than in the central part of the cross-section of the antherozoid. When, however, these same preparations are examined with the best apochromatic objectives, this effect is so much less noticeable as to suggest that it may be entirely optical.

I am unable to find in the antherozoids caught in fixed and sectioned archegonia any trace of the cilia or basal bodies or of the cytoplasmic vesicle. The cytoplasmic vesicle may possibly be lost before the antherozoid enters the archegonium, and the cilia, if present, could scarcely be expected to be visible after the differentiation of the stain. The body of the antherozoid undergoes no further visible change after becoming applied to the surface of the egg until it begins to penetrate the nucleus of the egg. Since this body of diminished thickness appears to consist only of the

<sup>1</sup> See, however, the third paper of this series, on fertilization in *Fossombronina*, to appear in the next number of this Journal.

nucleus of the antherozoid, it will henceforth be referred to as the male nucleus.

The application of the male nucleus to the surface of the egg seems to be simultaneous, or nearly so, throughout its whole length. Pl. XXV, Fig. 35, represents an egg with the applied male nucleus, in a plant which was fixed six minutes after insemination of the culture. There are fourteen eggs with male nuclei thus applied to their surfaces in the preparations of plants from this fixation. In none of them has an antherozoid or a male nucleus been found which is applied to the egg through only a part of its length; in some of these cases, however, the view is obscured by the supernumerary antherozoids, so that the full length of the male nucleus cannot be traced. In these same preparations are found three archegonia, each with one or more antherozoids in the venter, none of the latter, however, having yet applied itself to the egg. Each of two archegonia shows an antherozoid in the neck, and four contain eggs apparently functional, but no antherozoids. Two archegonia contain so many antherozoids about the egg that I cannot determine whether or not one of them has applied itself to the egg.

The application of the male nucleus to the egg is followed by a depression in the surface of the latter, so that the male nucleus comes to lie in a groove (Pl. XXV, Fig. 36). I am unable to determine with certainty whether or not the plasma membrane of the egg is continuous at the base of the groove and beneath the male nucleus. In material fixed 20 to 30 minutes after insemination, some cases show the male nucleus apparently in the surface membrane of the egg (Pl. XXV, Fig. 36) and others show that the cytoplasm of the egg has become continuous outside of the male nucleus (Pl. XXV, Fig. 37). I can detect no visible alteration of the plasma membrane of the egg accompanying or shortly following the penetration of the male nucleus. In this respect my observations are in harmony with those of Rickett (1928) on *Sphaerocarpos*, who shows that the so-called 'fertilization membrane' of earlier investigators is probably not at all analogous to the fertilization membrane of the animal egg.

Usually only one male nucleus becomes applied to the surface of an egg, although many antherozoids may crowd into the archegonium. It seems reasonable to infer that the first antherozoid to come into contact with the egg is the one which functions. I have found, however, in heavily inseminated plants, several eggs each with two male nuclei apparently in the process of penetration. In such a case two antherozoids may have reached the egg simultaneously, or the second may have applied itself very shortly after the first one reached the surface of the egg.

The supernumerary antherozoids in the archegonium, after about 18 hours, undergo, apparently quite suddenly, a reduction in diameter and change in staining capacity. In the same archegonium are found some which have undergone this change and some which have not. The reduced deeply

staining rods retain their form and staining capacity and are easily recognized surrounding the fifteen-day embryo.

I am unable to find in the literature any account of a similar fusion of gametes or penetration of the antherozoid into the egg, and the process is in need of more thorough study before a final interpretation is attempted. The following interpretation is offered as provisional. The apparently simultaneous application of the antherozoid throughout its full length to the surface of the egg is probably a phenomenon of surface tension. Surface tension is presumably much higher in the surface of water against either the cell membrane of the egg or against that of the antherozoid than it is in the surface of the two membranes against each other. When the surface film of the water is broken by the contact of the two membranes at one point, the surface tension of the water would force the antherozoid against the egg throughout its whole length. The reduction in thickness and alteration of staining capacity of the antherozoid simultaneously with its application to the surface of the egg suggests two possibilities with reference to the structure and reaction of the antherozoid. First, the body of the antherozoid may be impregnated with a substance which is readily soluble in the cytoplasm of the egg or its surface layer, and which is dissolved out immediately after coming into contact with the egg, leaving the deeply staining rod of less soluble substance (chromatin) on the surface of the egg. Second, and I think more probably, the body of the antherozoid may consist of a central nucleus surrounded by a cortex of non-nuclear substance which dissolves in the cytoplasm of the egg or its surface layer, causing a change in the physico-chemical reaction of the latter so that no further antherozoids are attracted to it. The furrowing of the surface film or membrane of the egg along the line of contact with the male nucleus may be due to an alteration of the surface tension, and the later passage of the nucleus through this film may be due to the same cause. Such a furrowing might also be due to a shortening of the male nucleus or, more probably, to growth in the egg.

#### *Fusion of Male and Female Nuclei.*

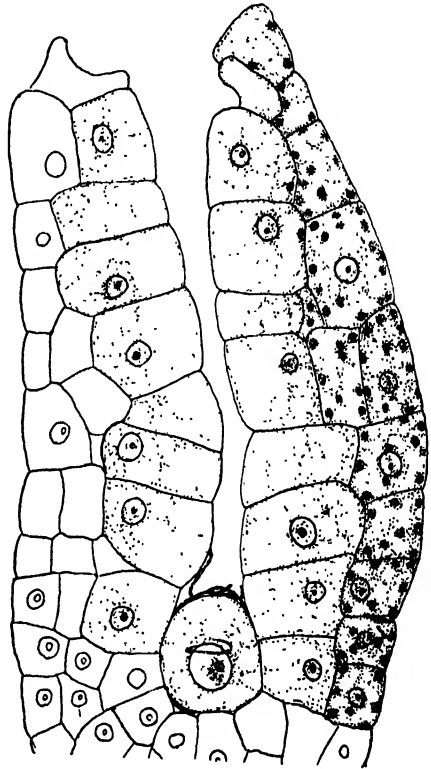
The male nucleus remains without perceptible change of form in the cytoplasm of the egg for from 24 to 36 hours. During this time changes occur in the egg and in the inner layer of cells of the archegonium. The fertilized egg grows rapidly; its cytoplasm becomes vacuolate and loses its capacity to stain darkly (Pl. XXV, Figs. 39-46). The chromatin of the female nucleus aggregates about the nucleole (Pl. XXV, Figs. 39-41). The cells of the inner layer of the archegonium enlarge, projecting inward, those of the venter compressing the egg laterally (Pl. XXV, Fig. 39) and those of the neck reducing or closing the canal (Text-fig. 2). This enlargement or growth of the cells of the inner layer of the archegonium seems not to be due entirely

to a stimulus resulting from fertilization, for old archegonia in which no fertilization has occurred sometimes show similar changes. The appearance of these cells about and above the fertilized egg suggests that they may function in the nutrition of the zygote. They contain few or no chloroplasts, and their protoplasm stains much less deeply than does that of the cells of the outer layer of the archegonium (Text-fig. 2). As a result of this growth of the cells of the interior the diameter of the archegonium is considerably increased (Text-figs. 1-3).

From 24 to 36 hours after insemination the male nucleus begins to penetrate the female nucleus. In the earliest stages of this penetration one end of the male nucleus is seen to project into the cavity of the female nucleus and to be much enlarged (Pl. XXV, Figs. 41, 42). This swollen end of the male nucleus becomes a vesicle of deeply staining material which increases in size as more of the rod-shaped male nucleus passes into the female nucleus (Pl. XXV, Figs. 42-46). This vesicle appears to be bounded by a definite membrane until nearly all of the male nucleus is within the cavity of the female nucleus. This endwise passage of the male nucleus through the membrane of the female nucleus is very slow and requires 20 to 30 hours for its completion. In the earlier stages of this penetration

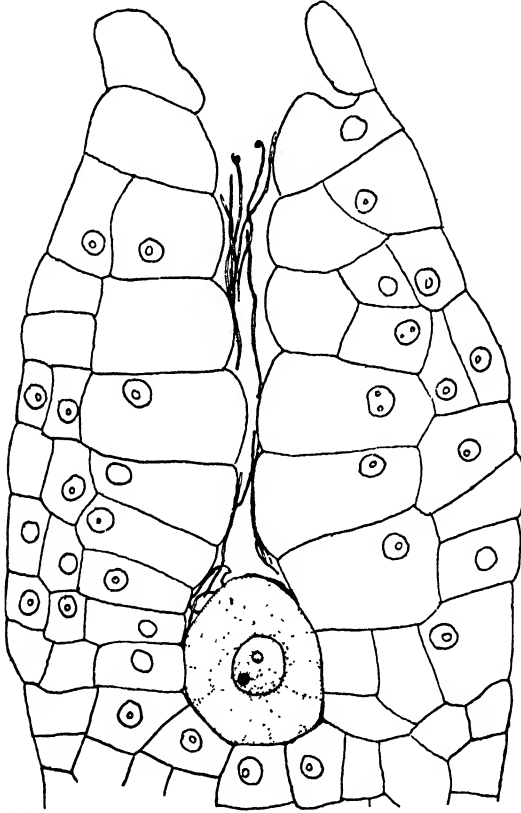
the aggregated maternal chromatin and the vesicle of incoming paternal chromatin are always in close juxtaposition (Pl. XXV, Figs. 42-44). During the later stages the maternal chromatin loosens up and comes to form a more or less irregular reticulum of tangled anastomosing threads, quite suggestive of the condition of the chromatin in some of the early prophase of mitosis (Pl. XXV, Figs. 43-46).

The paternal chromatin sometimes becomes optically heterogeneous in the vesicle (Pl. XXV, Figs. 46, 47) before the disappearance of the vesicular membrane, which disappearance seems to be abrupt. Immediately after this membrane has disappeared the paternal chromatin is recognizable as



TEXT-FIG. 2. Longitudinal section of archegonium and zygote, 18 hrs. 45 min. after insemination.  $\times 550$ .

a partially disperse granular mass more dense at its centre and without definite outer boundary. One or more nucleoles are present in the central region of this mass (Pl. XXV, Figs. 48, 49). At this time the paternal and maternal chromatin masses occupy separate general regions of the nuclear cavity, but there is no distinct plane of demarcation between the two regions, each of which contains at least one nucleole (Pl. XXV, Figs. 48-51). The paternal chromatin is less evenly distributed than is the maternal, and



TEXT-FIG. 3. Longitudinal section of archegonium and zygote, 60 hrs. 30 min. after insemination.  
× 550.

is less thread-like. These two regions of respectively less and more disperse chromatin are sometimes recognizable as late as four days after the time of insemination (Pls. XXV and XXVI, Figs. 52-56). The two regions, however, become indistinguishable before the beginning of the prophase of the first mitosis (Pl. XXVI, Figs. 57, 61, 63, 64). During the passage of the male into the female nucleus there appear in the nuclear cavity usually one to several small globular bodies of which only the surface layer is visibly stained (Pl. XXV, Figs. 45, 46, 49-52). The nature of these bodies has not been determined.



There are some cases in which the maternal chromatin fails to become loosened from its aggregation about the nucleole and in which it is therefore not distinguishable from the paternal chromatin (Pl. XXVI, Figs. 58, 59). It is possible that such zygotes are incapable of development, since a considerable proportion of zygotes fail to develop beyond the stage of one large cell, and some of these whose development is checked show a similar uneven distribution of the chromatin.

It was stated above that several cases were found which showed two male nuclei apparently in the process of entering the same egg. I have found but one conclusive case of dispermy in a later stage than those. This case is in a plant fixed  $41\frac{1}{2}$  hours after insemination, and shows the two male nuclei penetrating the female nucleus from opposite sides (one in upper, and one in lower focus, Pl. XXVI, Fig. 60).

The manner of the fusion of the male and female nuclei in *Riccardia* is radically different from anything previously described in plants or animals. The various fragmentary observations of stages of fertilization in the Marchantiales seem to indicate that the male nucleus becomes spherical with its chromatin in the condition characteristic of the resting nucleus before its union with the female nucleus. In *Sphaerocarpus* (Rickett, 1923) and *Riella* (Kruch, 1890) the male and female nuclei remain separate until each has organized its chromosomes in preparation for the first division of the zygote. In the ferns *Onoclea* (Shaw, 1898) and *Nephrodium* (Yamanouchi, 1908) the antherozoid, or at least its nucleus, passes bodily and without previous alteration into the egg nucleus immediately after its entrance into the egg.

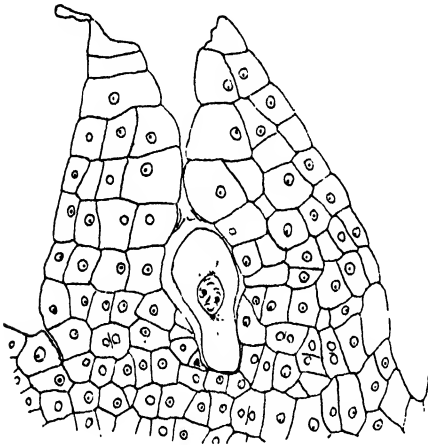
#### *The First Segmentation of the Zygote.*

The fertilized egg grows continuously from the time of the entrance of the antherozoid; but its rate of growth after the first 36 hours is less noticeable until the last 24 hours preceding the first cell division, which occurs from six to nine days after fertilization. About 24 hours before this division the zygote begins to elongate at its basal end, forming a projection which penetrates the mass of small, rapidly multiplying cells at the base of the archegonium (Pl. XXVI, Figs. 61, 62, and Text-fig. 4). The zygote now has increased greatly in volume, and its cytoplasm contains large vacuoles (Pl. XXVI, Fig. 62). Fixation is attended with considerable shrinkage of the zygote away from the wall of the cavity in which it lies (Pl. XXVI, Figs. 61, 62, 65, 67, 68, and Text-fig. 4). There are few stainable bodies in the cytoplasm and the granular substance which forms the continuous phase separating the vacuoles stains very lightly. The nucleus has the structure of the typical 'resting' stage with the chromatin reticulum evenly distributed throughout the nuclear cavity, and with one or more nucleoles

(Pl. XXVI, Figs. 61, 63). No trace of distinction between the maternal and the paternal chromatin is visible in these stages.

The first indication of a preparation for nuclear division is a change in the structure of the chromatic reticulum. This reticulum becomes coarser and more definitely thread-like (Pl. XXVI, Figs. 64, 66). Almost simultaneously with these changes the granular cytoplasm begins to aggregate in two regions just outside the nucleus towards the ends of the zygote (Pl. XXVI, Fig. 65). The granular continuous phase of the cytoplasm forms a radiate configuration about the two centres, and the vacuoles are laterally compressed between the radiations (Pl. XXVI, Figs. 65, 67, 68). No centrosomes have been found in the cytoplasmic aggregations nor later at the poles of the spindles.

The nucleus, which at first is quite spherical (Pl. XXVI, Fig. 66), soon



TEXT-FIG. 4. Longitudinal section of archegonium and zygote, 148 hrs. after insemination.  $\times 275$ .

elongates in the direction of the long axis of the zygote, its two ends abutting against the aggregations of cytoplasm (Pl. XXVI, Figs. 67, 68). During the time of the cytoplasmic migrations the threads of the nuclear reticulum become shorter and thicker until the chromosomes are definitely recognizable (Pl. XXVI, Figs. 66-68). No continuous spireme has been found. The chromosomes become condensed into smooth, slender rods which are seen to be of the diploid number (20) and which, until the disappearance of the nuclear membrane, are fairly evenly distributed throughout the nuclear cavity. Ap-

parently the disappearance of the nuclear membrane is simultaneous with the appearance of the achromatic spindle, and the migration of the chromosomes to the equatorial plane of the spindle seems to follow very shortly. I have found no stage intermediate between that represented by Pl. XXVI, Fig. 68, and that represented by Fig. 69. At about the same time the radiations in the cytoplasm disappear, but the two masses of dense granular cytoplasm remain in the same positions. The poles of the achromatic spindle are in these two masses (Pl. XXVI, Fig. 69). Each pole is single, and no distinction between maternal and paternal chromosomes is discernible. The longitudinal halves of the chromosomes separate (Pl. XXVI, Fig. 70) and pass to the poles of the spindle (Pl. XXVI, Fig. 71). The cell plate begins to be formed in the centre of the spindle and

extends outward, following the formation of additional spindle strands, until cell division is complete (Pl. XXVI, Fig. 71; Pl. XXVII, Fig. 72).

The polar aggregations of granular cytoplasm remain prominent during the division of the cell and for some time thereafter (Pl. XXVII, Figs. 72, 73). The substance of these two masses gradually becomes dispersed until the granular cytoplasm is uniformly distributed in the two cells (Pl. XXVII, Fig. 74).

These changes in the configuration of the granular cytoplasm accompanying nuclear and cell division in the zygote are not essentially different from those accompanying nuclear and cell division in the cells of the thallus, an account of which will be published later.

It happens frequently that the eggs of more than one archegonium on the same archegonial branch are fertilized simultaneously. The resultant zygotes develop apparently at the same rate to a stage just preceding the sudden downward elongation of the zygote. Usually only one zygote in the branch elongates and continues to develop. The other or others remain spherical and do not undergo division. I have found two cases, however, in each of which two zygotes in the same branch had elongated and had apparently continued normal development. In one of these cases the nucleus of one of the two zygotes is in prophase (Pl. XXVI, Fig. 68) and that of the other zygote has just divided (Pl. XXVII, Fig. 73). In the other case two apparently normal two-celled embryos are present in the same branch. Since, in this species, the whole archegonial branch is transformed into a 'calyptra' with the developing sporophyte at its centre, it seems probable that two embryos in the same branch, if both continue to develop, would come together at least in their basal regions and occupy a common 'calyptra'. Coker (1907) has reported a case of two mature sporophytes in the same 'calyptra' in this species.

#### *Early Embryogeny.*

During the two days preceding the first segmentation of the zygote the cells in the base of the archegonium and those immediately beneath multiply rapidly, forming a mass of small cells with relatively large nuclei and dense cytoplasm. The downward projection of the base of the zygote forms a haustorium which penetrates this tissue, destroying the cells in its path. The length of the haustorium at the time of the first sporophytic division is usually two or three times its diameter (Pl. XXVII, Figs. 72, 74); but in some cases the haustorium is short and wide (Pl. XXVII, Fig. 73). The first division of the zygote results in a hypobasal or haustorial cell and an epibasal cell; this latter gives rise to the embryo proper (cf. Clapp, 1912). The haustorial cell elongates farther and sometimes branches, but undergoes no division (Pl. XXVII, Fig. 76). The epibasal cell enlarges and divides

transversely, forming a filament of three cells—including the haustorium (Pl. XXVII, Figs. 75, 76). The next division occurs in the middle cell and is vertical (Pl. XXVII, Figs. 76, 77). The following division in the terminal cell was not observed, but later stages indicate that it is probably vertical.

Archegonia, each with a lateral opening in the venter, are occasionally found in this species. An interesting deviation from the usual manner of embryo development is represented by a two-celled embryo in such an archegonium (Pl. XXVII, Fig. 78). The embryo in this case has elongated in a direction transverse to the long axis of the archegonium and extends towards the lateral opening of the archegonium. The two cells formed by the division of the zygote show no differentiation and neither of them has the appearance of a haustorium. The cells of the thallus in contact with the zygote in the position usually occupied by a haustorium show some evidence of being destroyed, and the zygote bulges slightly on this side. Supernumerary antherozoids in the lateral opening of the archegonium as well as in the neck canal indicate that both these openings existed at the time of insemination.

#### SUMMARY.

1. The penetration of the antherozoid into the egg is a gradual process requiring from 20 to 30 minutes for its completion. The antherozoid applies itself to the surface of the egg and is immediately reduced in thickness by 50 to 70 per cent. Apparently only the nucleus of the antherozoid enters the egg.

2. The male nucleus remains for 24 to 36 hours without perceptible change of form in the cytoplasm of the egg. During this time the egg increases greatly in size and the chromatin of its nucleus becomes aggregated about the nucleole.

3. The male nucleus penetrates endwise and very gradually into the female nucleus. The chromatin of the female nucleus, previously aggregated about the nucleole, loosens up and becomes somewhat thread-like.

4. The maternal and paternal chromatin are distinguishable for about two days after the union of the two nuclei. They become indistinguishable, however, before the prophases of the first mitosis.

5. The zygote increases greatly in volume and produces a haustorium before its first segmentation.

6. The early development of the embryo is described briefly.

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EXPLANATION OF PLATES XXV-XXVII.

Illustrating Dr. Showalter's paper on Fertilization in *Riccardia pinguis*.

All figures were drawn with the aid of the Abbé camera lucida at the magnification indicated and are reproduced without reduction.

The numbering of the figures runs on from Plate XXIV of the previous paper (pp. 691-707).

To indicate the time elapsed between insemination and the fixing of the preparation the symbol ° is used for hours and ' for minutes.

PLATE XXV.

Fig. 35. Antherozoid applied to surface of the egg, 0° 06' after insemination. × 2,100.

Fig. 36. Egg showing the male nucleus in its surface, 0° 23' after insemination. × 2,100.

Fig. 37. Egg showing the male nucleus inside of its plasma membrane, 0° 23' after insemination. × 2,100.

Fig. 38. Egg showing the male nucleus in its cytoplasm, 4° 30' after insemination. × 2,100.

Fig. 39. Egg showing the male nucleus in its cytoplasm, 18° 45' after insemination. × 2,100.

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- Fig. 40. Egg showing the male nucleus in its cytoplasm,  $18^{\circ} 45'$  after insemination.  $\times 2,100$ .  
 Fig. 41. Egg showing the male nucleus with one end projecting into the female nucleus,  $28^{\circ} 30'$  after insemination.  $\times 2,100$ .  
 Fig. 42. Egg showing the male nucleus with one end in female,  $34^{\circ} 30'$  after insemination.  $\times 2,100$ .  
 Fig. 43. Egg showing the male nucleus about half inside the female,  $34^{\circ} 30'$  after insemination.  $\times 2,100$ .  
 Fig. 44. Egg showing the male nucleus almost entirely within the female,  $46^{\circ} 45'$  after insemination (section transverse to long axis of archegonium).  $\times 2,100$ .  
 Fig. 45. Egg showing the male nucleus almost entirely within the female,  $46^{\circ} 45'$  after insemination.  $\times 2,100$ .  
 Fig. 46. Egg showing the male nucleus almost entirely within the female,  $60^{\circ} 30'$  after insemination.  $\times 2,100$ .  
 Fig. 47. Male nucleus almost entirely within the female,  $41^{\circ} 30'$  after insemination.  $\times 2,100$ .  
 Fig. 48. Male nucleus almost entirely within the female,  $53^{\circ} 30'$ .  $\times 3,200$ .  
 Fig. 49. Male nucleus almost entirely within the female,  $53^{\circ} 30'$ .  $\times 3,200$ .  
 Fig. 50. Fusion nucleus, maternal chromatin at the right, paternal at the left, part of the latter still rod-shaped,  $53^{\circ} 30'$ .  $\times 3,200$ .  
 Fig. 51. Same as Fig. 50.  
 Fig. 52. Fusion nucleus, paternal chromatin in low focus at centre less disperse or thread-like than the maternal above,  $60^{\circ} 30'$ .  $\times 2,100$ .  
 Fig. 53. Fusion nucleus, maternal chromatin at left, paternal at right,  $60^{\circ} 30'$ .  $\times 2,100$ .

PLATE XXVI.

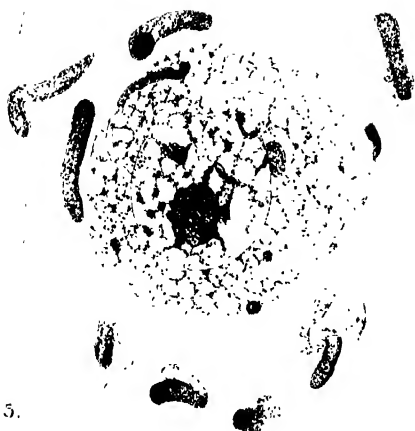
- Fig. 54. Fusion nucleus showing paternal chromatin at left, maternal at right,  $60^{\circ} 30'$ .  $\times 2,100$ .  
 Fig. 55. Fusion nucleus, paternal chromatin at left, maternal at right,  $77^{\circ}$ .  $\times 3,200$ .  
 Fig. 56. Fusion nucleus showing two regions of different degrees of dispersion,  $96^{\circ} 30'$ .  $\times 2,400$ .  
 Fig. 57. Fusion nucleus, maternal and paternal chromatin indistinguishable,  $96^{\circ} 30'$ .  $\times 2,100$ .  
 Fig. 58. Fusion nucleus, maternal and paternal chromatin both aggregated and indistinguishable,  $96^{\circ} 30'$ .  $\times 2,100$ .  
 Fig. 59. Fusion nucleus, maternal and paternal chromatin both aggregated and indistinguishable,  $60^{\circ} 30'$ .  $\times 2,400$ .  
 Fig. 60. Two male nuclei entering the same female nucleus,  $41^{\circ} 30'$ .  $\times 2,100$ .  
 Fig. 61. Zygote with haustorium,  $120^{\circ} 30'$ .  $\times 1,330$ .  
 Fig. 62. Zygote with haustorium just before prophase of first mitosis,  $152^{\circ}$ .  $\times 1,330$ .  
 Fig. 63. Nucleus of same.  $\times 2,400$ .  
 Fig. 64. Zygote nucleus in very early prophase, only peripheral part of one hemisphere shown; rest of cell in same condition as that shown in Fig. 62;  $152^{\circ}$ .  $\times 2,400$ .  
 Fig. 65. Very early prophase of first mitosis, aggregations of granular cytoplasm in two regions near nucleus,  $152^{\circ}$ .  $\times 1,665$ .  
 Fig. 66. Nucleus of zygote shown in Fig. 65.  $\times 2,400$ .  
 Fig. 67. Later prophase of first mitosis,  $148^{\circ}$ .  $\times 1,900$ .  
 Fig. 68. Still later prophase of first mitosis (section slightly oblique),  $152^{\circ}$ .  $\times 1,900$ .  
 Fig. 69. Metaphase figure, first mitosis,  $152^{\circ}$ .  $\times 1,900$ .  
 Fig. 70. Anaphase figure, first mitosis,  $191^{\circ}$ .  $\times 1,900$ .  
 Fig. 71. Telophase figure, first mitosis,  $148^{\circ}$ .  $\times 1,900$ .

PLATE XXVII.

- Fig. 72. Late stage of first cytokinesis in zygote,  $152^{\circ}$ .  $\times 1,900$ .  
 Fig. 73. Zygote just after first division,  $152^{\circ}$ .  $\times 1,000$ .  
 Fig. 74. Two-celled embryo,  $191^{\circ}$ .  $\times 1,000$ .  
 Fig. 75. Two-celled embryo,  $245^{\circ}$ .  $\times 1,000$ .  
 Fig. 76. Three-celled embryo,  $245^{\circ}$ .  $\times 1,000$ .  
 Fig. 77. Diagram of four-celled embryo.  
 Fig. 78. Two-celled embryo in aberrant archegonium,  $191^{\circ}$ .  $\times 195$ .



35.



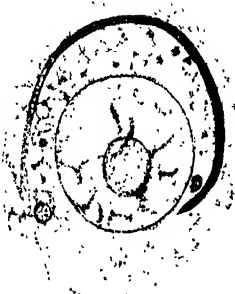
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36.



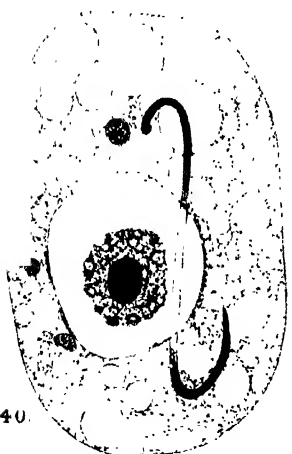
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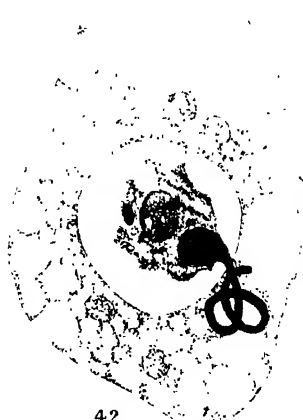
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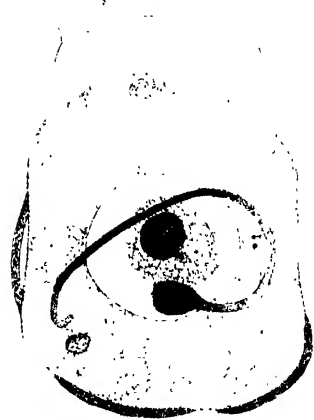
40.



42.



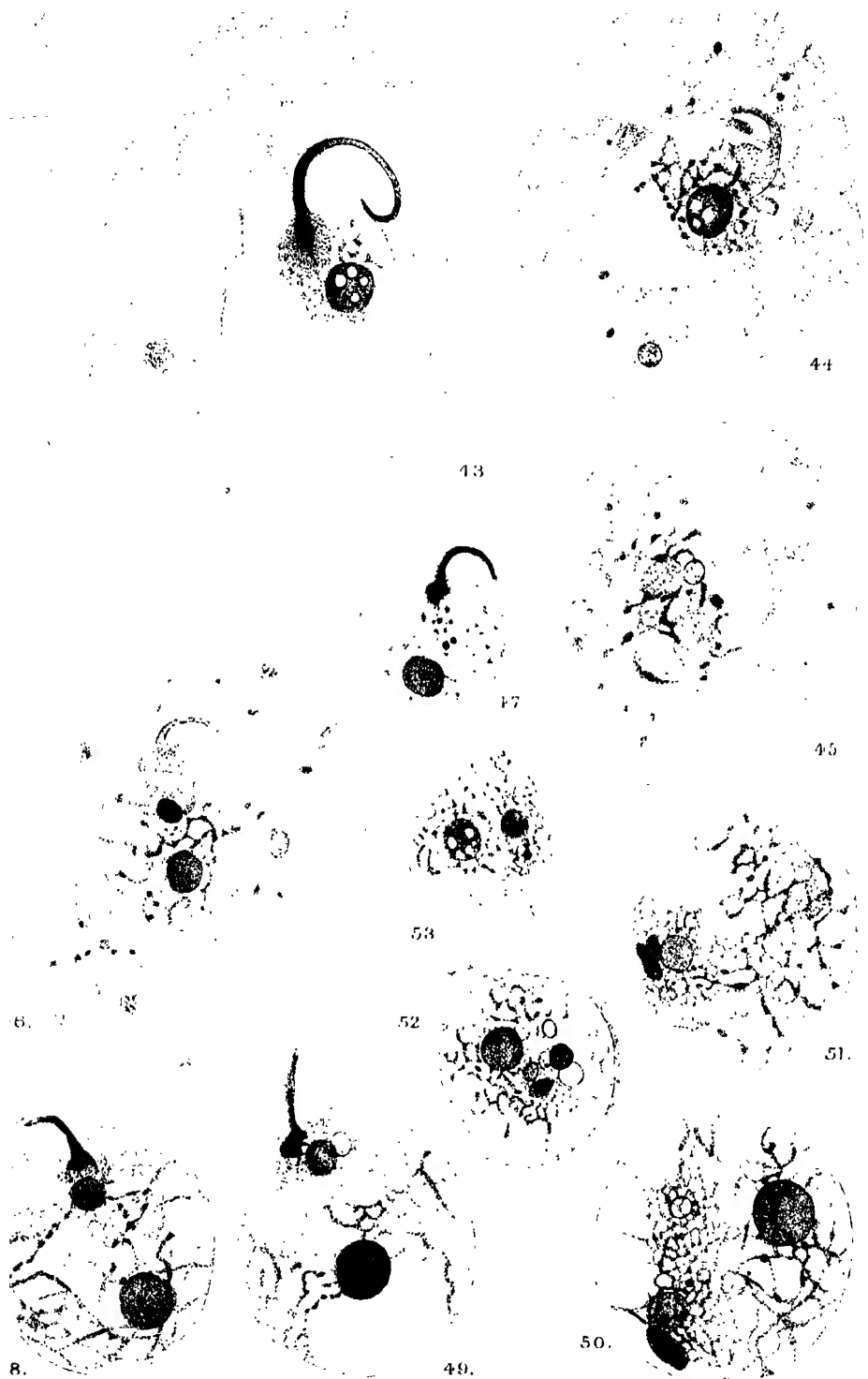
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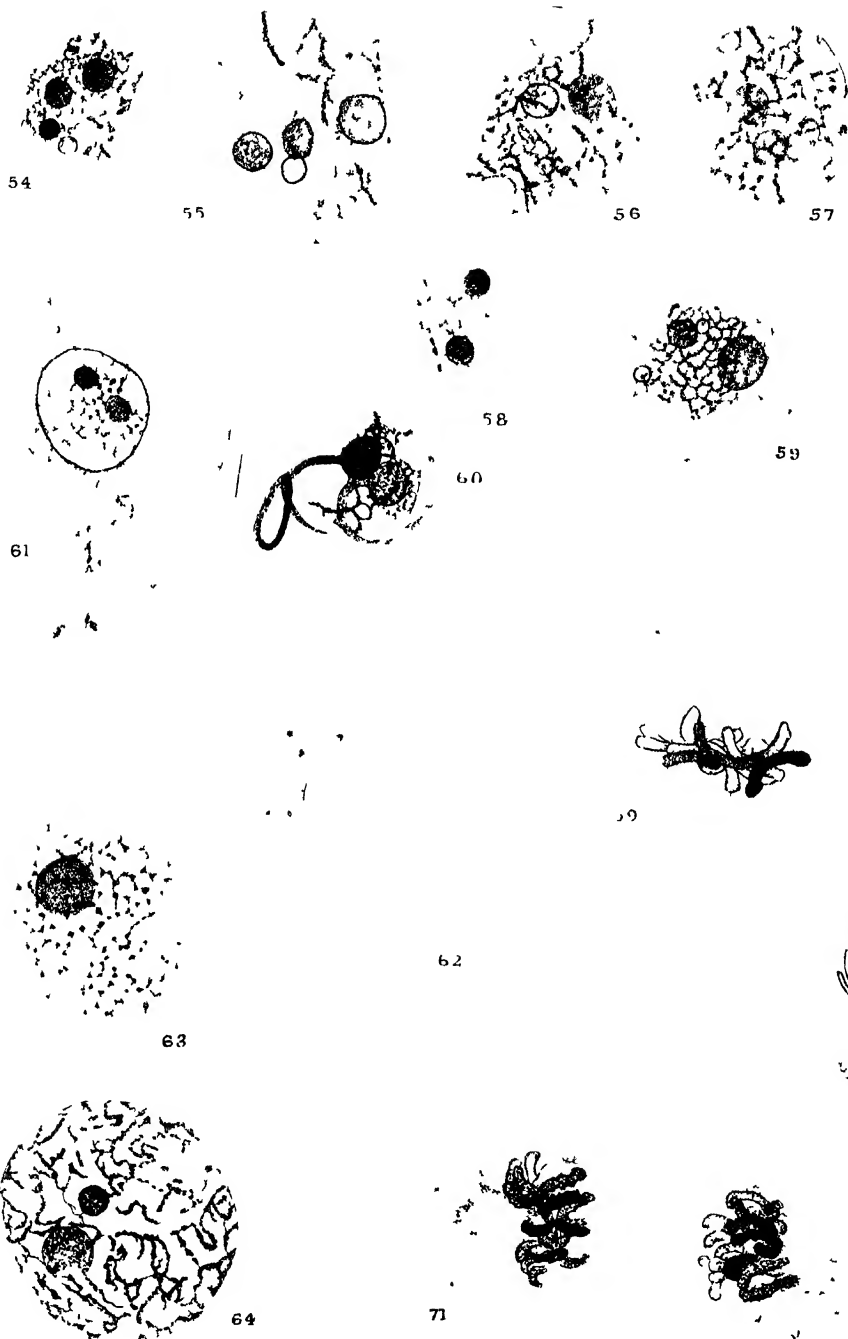


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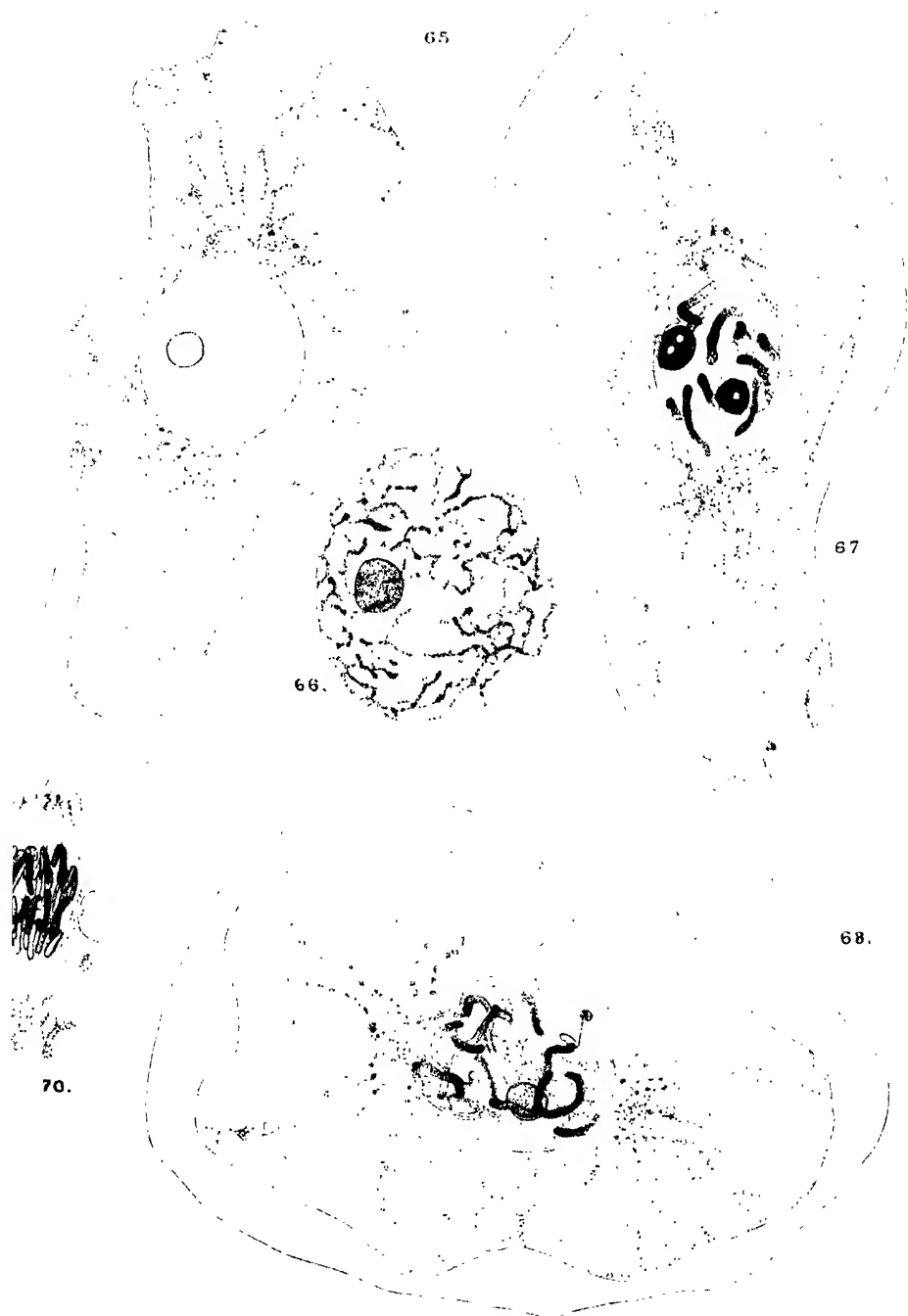








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Huth, London



# The Structure of *Peltigera* with Especial Reference to *P. praetextata*.

BY

O. V. DARBISHIRE.

With Plates XXVIII-XXXI.

THE main object of this paper is to give an account of the structure and development of the isidia of *Peltigera praetextata*, (Flk.) Zopf, but in order to make this clear it is first necessary to describe the tissues found in the young protothallus and in the mature metathallus. A description of the structure of the rhizines is included in this paper for the same reason.

As most of the species of *Peltigera* are very much alike in regard to structure, I have, for the purposes of this investigation, made a study of some species other than *P. praetextata*, when the material from these other species appeared to be more suitable. The species to which reference is made in the text are the following:

*Peltigera canina*, Willd., Hue (12, No. 361); A. Zahlbr. (37, No. 6230).

*Peltigera canina*, Willd., f. *spongiosa*, Tuck., Tuck. (36, p. 109); A. Zahlbr. (37, No. 6230 f).

*Peltigera horizontalis*, Baumg., Hue (12, No. 364); A. Zahlbr. (37, No. 6237).

*Peltigera lepidophora*, (Nyl.) Bitter, Bitter (3, p. 251); A. Zahlbr. (37, No. 6238).

*Peltigera polydactyla*, Hoffm., Hue (12, No. 363); A. Zahlbr. (37, No. 6245).

*Peltigera praetextata*, (Flk.) Zopf., Hue (12, No. 361) (var. of *P. canina*); A. Zahlbr. (37, No. 6249) (var. of *P. rufescens*, Humb.); Linkola (15, pp. 65 and 77); du Rietz (23, p. 210).

In my experience it is easy to separate these species from one another. *P. praetextata* alone among British species normally produces true upright isidia. The isidia formed by *P. lepidophora* are much smaller than those of *P. praetextata*, and more closely pressed to the thallus, but *P. lepidophora* is not known from Great Britain. Cracks occur in the three other species,



but they heal up without apparently forming isidia. The cells of the mature cortex of *P. canina* are generally more rounded at their corners than those of *P. praetextata*. These two species as a rule have well-differentiated veins, which are not so clearly marked in *P. polydactyla* and *P. horizontalis*. The surface of the upper side of these two is also smoother, and the medullary hyphae are thicker. The apothecia are fixed horizontally to the thallus at its margin in the case of *P. horizontalis*.

Du Rietz has published a good review of the sorediiferous and isidiiferous species of *Peltigera*. He distinguishes the following species: *P. lepidophora*, (Nyl.) Bitt., *P. praetextata*, (Flk.) Zopf., *P. erumpens*, (Tayl.) Lang, and *P. scutata*, (Dicks.) Flot.; the two former are capable of producing isidia only, the two last soredia only. *P. canina* does not show either soredia or isidia (23, pp. 212–16). Birger Nilson, on the other hand, points out that the appearance of isidia and soredia is a purely biological phenomenon and depends on accidental external conditions. For that reason it is of no value in making species. The manner of this appearance, however, varies with the degree of moisture present and with the structure of the particular lichen, so their presence may be used as a supplementary character in separating species (20, p. 20). Moreau looks upon all isidiiferous and sorediiferous species, such as *f. crispata*, *undulata*, *crispa* and *propagulifera*, *sorediata* and *erumpens*, as mere habitat forms (18, p. 115).

Not a few of the points described in the following paper in detail have been touched on by other authors previously. In several cases, however, the latter have recorded their observations incompletely. In one or two instances the incompleteness is apt to mislead the reader.

The material used for investigation was as far as possible freshly gathered and then kept living till required. To see at any time whether the material was in a living condition or not, a small square piece was cut out and placed in the moist chamber of a Petri dish on some soil. If it were still living the medullary hyphae would grow out in a few days.

I am very much indebted to Dr. Linkola (Helsingfors) and Dr. Watson (Taunton) for sending me fresh material in a living condition. Some of this has been kept growing in cultures for nearly twelve months. I am indebted also to Mr. R. Paulson and other friends for supplying me with living and dead *Peltigera* material. I have to thank the Department of Scientific and Industrial Research for enabling me to employ a whole-time laboratory assistant for preparing and cutting my material, the Colston Research Society for a grant towards the cost of publishing this paper, and my wife for valuable assistance rendered in the course of its preparation.

For gross anatomical work fresh material in a moist condition was fixed in absolute alcohol, dehydrated, and passed through cedar-wood oil into hard paraffin and then cut with a microtome. Sections were mounted in glycerine jelly to which had been added a small amount of gentian violet.

*The Structure of the Mature Metathallus.*

The structure of the mature metathallus of *Peltigera* has been described previously by various authors, as Speersschneider (32, p. 521), Schwendener (27, p. 174), Strato (34, p. 14), Hue (12, No. 360), Moreau (18, p. 35), and others. I have referred to these in a former paper (8, p. 17), and I have there made some necessary criticisms. I am here merely recalling what has already been said on the matter and adding a few details for the sake of greater accuracy.

The following tissues can be distinguished in the case of *P. praetextata*, which I have chosen as a typical example (Pls. XXVIII, XXIX, Figs. 1 and 20): (1) The cortex on the upper side with occasional epicortical hairs, (2) the gonidial layer, (3) the intergonidial hyphae, (4) the infragonidial hyphae, (5) the medullary hyphae, (6) the hypothallus, and (7) the rhizines. The depth and general development of the different tissues varies according to their distance from the protothallus, and to some extent probably with the ecological conditions, as does the thickness of the walls.

The cortex in the mature metathallus is two to four cells deep; the cells vary very much in size and shape. They are angular in outline and thus differ from those of *P. canina*, which are more rounded. The walls of the latter are therefore of more varying thickness. With *P. praetextata* the walls separating two neighbouring cortical cells are uniformly about  $1.5$  to  $2\ \mu$  thick, the wall of each cell thus having a thickness of about  $0.75$  to  $1\ \mu$ . In diameter the cells vary from  $10$  to just under  $20\ \mu$ , and in height measure up to  $24\ \mu$ . At the corners there is occasionally a slight thickening, and the outer protective walls are  $2$  to  $3\ \mu$  thick. The cells are always to be seen in rows, and they have living contents. In the mature metathallus they have lost the power of renewed growth should they be injured in any way. Since the cells are closely cemented together the cortical layer is characterized by a complete absence of intercellular air-spaces, except where it is in touch with the gelatinous envelope of the gonidia. Small interhyphal air-spaces can be noticed at this point.

The innermost cells of the cortex narrow down very much at their inner ends, which are connected with the delicate intergonidial hyphae. The broadest of these measure  $5$  to  $6\ \mu$  in diameter, and they pass more or less directly into the tissues below. From the broader hyphae branches of smaller diameter pass in between the folds of mucilage, and some even enter the mucilaginous sheath of the gonidia. The intergonidial hyphae proper vary much in shape, but their diameter rarely exceeds  $3\ \mu$ . The walls of all the intergonidial hyphae are very thin. Air-spaces are found between these hyphae and the outer mucilaginous sheath of the gonidia. But although these air-spaces are extensive their diameter is always very

small. In the gonidial layer hyphae are distributed in the following manner. There are first the larger hyphae which are, so to say, on their way from the hypothallus or from the medulla to the cortex. They run between the largest groups of the algal colonies. As a single algal colony I designate the small sausage-shaped mucilaginous mass inside which is found a single *Nostoc* thread. Between these colonies we get branches from the larger hyphae just mentioned and others coming directly from the upper medullary hyphae. From these branches there arise further hyphae which penetrate between the folds of the mucilaginous sheath. All these hyphae show extensive fusions, so that all together they form a firm network. It is impossible to say in any particular case whether a connexion is due to a primary branching or to a secondary fusion. I suspect that most of the connexions with the hyphae which run directly from hypothallus to cortex are secondary. Very occasionally still finer hyphae are found which have actually penetrated into the mucilage surrounding the gonidia. I have never observed hyphae which have actually entered a *Nostoc* cell. Between the gonidial mucilage we have, therefore, a network of fungal cells which must be in close osmotic touch with the algal cells.

Immediately below the gonidial layer we find for a varying distance a number of hyphae which are in touch with the system of intergonidial hyphae on the one hand, and the longitudinally running medullary hyphae on the other. In *P. praetextata* these longitudinal hyphae are closely united in bundles or veins. These veins run longitudinally, but at the same time their course is wavy and they appear to branch. They are really connected with one another, very much like the bundles of sclerenchymatous cells in the hard bast of the lime tree.

Between the veins are found very much twisted and loosely packed hyphae. These form the loose tissue by which the gonidia are in touch with the outside air. These hyphae will not take up any moisture at all when placed in water. The air-passages can easily be traced right up to the cortex by mounting a section of fresh material in glycerine or some such medium. These gaps in the tissues of the under side are covered by the hypothallus, as is the whole remaining under surface of the metathallus. The hypothallus will not take up water when placed in it. The gaps between the veins originate and grow in size owing to the intercalary growth of the metathallus, with which the veins as such do not keep pace in a lateral direction, as Bitter points out (2, p. 141).

Meyer publishes a figure by Salter (17, Pl. VI, Fig. 6) in which the connexions of the various fungal constituents of the *Peltigera* thallus are well shown, though only diagrammatically. The net-like arrangement of the intergonidial hyphae is brought out well (see also id., Pl. VI, fig. 11).

A comparison of the cells of the metathallus with those of the protothallus is instructive and is given here:

	<i>Protothallus.</i>	<i>Metathallus.</i>
Depth of cortical layer . . .	20-25 $\mu$ .	30-40 $\mu$ .
Outer wall of cells . . .	2-3 $\mu$ .	2-3 $\mu$ .
Cells in each row . . .	5-6 $\mu$ .	2-4 $\mu$ .
Height of each cell . . .	8-12 $\mu$ .	up to 24 $\mu$ .
Diameter . . .	4-5 $\mu$ .	5-18 $\mu$ .
Inner walls . . .	1 $\mu$ .	1.5-2 $\mu$ .
Cells in 100 $\mu$ . . .	18-20 $\mu$ .	about 10 $\mu$ .
Depth of gonidial layer . . .	30 $\mu$ .	60-80 $\mu$ .
Gonidia in diameter . . .	4-6 $\mu \times 2-3 \mu$ .	8-10 $\times 4-6 \mu$ .
Depth of mucilage . . .	1-2 $\mu$ .	2-3 $\mu$ .
Hyphae between groups . . .	2-3 $\mu$ .	5-6 $\mu$ .
„ „ colonies . . .	1-2 $\mu$ .	3 $\mu$ .
Depth of infragonidial layer . . .	10 $\mu$ or so	100 $\mu$ or so
Diameter of hyphae . . .	3 $\mu$ .	4-6 $\mu$ .
Thickness of walls . . .	1 $\mu$ .	1.5 $\mu$ .
Depth of medulla . . .	20 $\mu$ .	120-150 $\mu$ .
Longitudinal hyphae, diameter . . .	2-3 $\mu$ .	8-10 $\mu$ .
Length of cells . . .	15-20 $\mu$ .	?
Thickness of walls . . .	0.5 $\mu$ .	1.5-2 $\mu$ .
Depth of hypothallus . . .	30-40 $\mu$ .	80-100 $\mu$ .
Diameter of hyphae . . .	6-7 $\mu$ .	5-6 $\mu$ .
Thickness of walls . . .	2 $\mu$ .	2 $\mu$ .

These figures show to what the increase in surface area of the thallus is due. In the cortical layer some of the outer cells with the epicortical hairs have been thrown off, so that we get fewer cells in each row, yet the cortex becomes thicker owing to the increase in length of the separate cells. These also increase in diameter, though they still remain cylindrical in form. The increase in surface area due to the cortical cells is so great that one square mm. of thallus surface at the growing margin of the thallus becomes four square mm. in the mature thallus. Increase in thickness of the thallus is mainly due to active growth in the medullary layer. The hyphae grow to five times their original thickness, but the whole medullary layer of longitudinal hyphae may become six to eight times thicker owing to a very large number of branches from other hyphae having pushed their way in. The medullary hyphae always retain the power of growth. The cortical cells, with possibly the outer hypothallial cells, lose this power. Schwendener also considers that the increase in surface is due to intercalary growth to a far greater extent than to marginal growth. At the same time the former occurs only in the very youngest parts of the metathallus (27, p. 174).

*Origin of the Tissues in the Protothallus of P. praetextata.*

The meristem in the case of *P. praetextata* is found just within the actual margin of the thallus, which represents the most advanced limit of the immature protothallus (Pl. XXIX, Figs. 19 and 20).

The *Nostoc* gonidia, surrounded in threads by the conspicuous mucilaginous sheath, extend up to this point. At first the mucilage is quite clear

and free from any close association with fungal hyphae. There is no evidence that the gonidia are formed by budding externally or by internal differentiation from the fungal constituent of the lichen in the protothallus. The mucilage clearly separates the algal cells from even the nearest fungal hyphae. It is reasonable to suppose that growth among the *Nostoc* cells is most active in this part of the thallus. Elfving maintains that in the lichens examined by him (and these include *Peltidea aphthosa* and *Peltigera canina*) gonidia and lichen-fungus are genetically connected, the former having originated within the hyphae of the latter, though at the same time the gonidia once formed can go on multiplying, so that each single *Nostoc* cell need not have originated in a fungal phypha. No clear evidence has, according to Elfving, been brought forward against his view (10, p. 56). He therefore rejects Schwendener's theory of the dual nature of the lichen (id., p. 58), and holds that certain algae when they represent escaped gonidia are descended from lichens (id., p. 59). He refers at length to *P. canina* (id., p. 55). Though he has made no direct observations in support of his view in this particular case, he believes that in *P. canina* the gonidia arise from the hyphae at a very early stage in the development of the thallus, and that all gonidia found in the older thalli have come from these first initial ones by division (id., p. 55). It is not clear to me, however, whether Elfving means the margin or protothallus of the lichen by 'early stage', or a still earlier stage when the thallus was still quite undifferentiated, namely, shortly after the germination of the fungal spore. Bearing Elfving's point of view in mind, I have examined the relation of algae to fungus near the growing-point very carefully and can find nothing to support his views.

Just below the gonidial layer hyphae generally run out in a direction at right angles to the margin. Beyond the last gonidia they bend upwards and branch, forming on the outside at first two or three more or less continuous layers of cells with very thick walls. Some of the cells, by growing out, help to form the tomentum by which the young margin is covered. These branch outwards in all directions and anastomose freely, increasing considerably in size and wall-thickness. Others continue to curve round the gonidia and grow back over the whole gonidial layer, and even over the cortex, which is now being differentiated between them and the gonidial layer. The inner cells of these rows are thin-walled, and it is these which in due course give rise to the cortex. Some of them continue in a curve like the outer hyphae; others seem not to grow beyond the outer level of the future cortex, and are not in direct touch with the tomental cells. Strato (34, p. 16) has already pointed out that the tomentum is formed near the margin only during the increase in area of the cortex. I find that it becomes loosened and may be thrown off.

The longitudinal hyphae below the gonidial layer not only give rise to the young cortex and its covering tomentum, but also to the tomentum

below, which ultimately becomes differentiated as the hypothallus. Extensive right-angle fusions may be observed in this region like those to be described later in the case of the rhizines. Fresh branches continually arise from the longitudinal hyphae which pass into the cortex between the groups of gonidia. From these ultimately arise the delicate hyphae which either enter the mucilage of the gonidia or merely encircle the groups of gonidia. The new cortical hyphae formed in this way do not seem to add to the epicortical tomentum by growing out beyond the general level of the cortical layer, though it is possible that they may do so near the margin. They appear at any rate to anastomose with some of the epicortical hairs from the margin. Near the margin of the thallus, vertical hyphae, which become part of the cortex, grow up from some of the longitudinal hyphae from which the hypothallus arises. These do not seem to get into very close touch with the intermediate hyphae of the medulla, which lie just below the gonidial layer, either by fusion or by contact. They simply connect up the hypothallus with the cortex. In the end the cortex is made up of those hyphae which are connected with the hypothallus and those connected more directly with the medulla and with the intergonidial hyphae.

From all this it will be seen that the ends of the longitudinal hyphae of the medulla really form the meristematic tissue of the protothallus, from which arise the hypothallus below, the cortex and intergonidial hyphae above, and the medullary hyphae of the metathallus, while the tomentum is developed at the margin to protect the more delicate meristematic plectenchyma.

Incidentally, in a foot-note, Bitter refers to the marginal growth in the thallus of *Peltigera*. But he considers, in agreement with Schwendener (27, p. 174), that both medullary and cortical hyphae branch at the margin, and thus increase the tissues there (2, p. 130). In my experience, however, a hypha, once it has become differentiated by division into a row of cortical cells, very rarely branches again, if ever. So marginal growth previous to even the simplest differentiation is due to the activity, branching, and elongation of the medullary hyphae only. In some species the medullary hyphae which run longitudinally become differentiated as veins or bundles of hyphae, which have already been referred to. In other species such veins are not differentiated. In any case gaps which have already been referred to are formed on the under side.

*Structure and Development of the Rhizines (Pl. XXVIII, Figs. 1 to 15).*

Rhizines are found on the under side of the various species of *Peltigera* to which reference has been made in this paper. They connect the main thallus with the substratum.

The structure of the rhizines of *P. canina* is typical of that of the rhizines of the genus generally (Figs. 12 to 15). They are usually white, though when old they may assume a darker coloration. They occur in connexion with the veins and actually arise, close to the growing margin, as small conical bundles of short hyphae. The hyphae of the rhizines form a system strictly lateral to, but connected with, the hyphae of the veins. The young rhizine seems to push its way through the hypothallus, and it grows chiefly by its apex.

The growing-point of the rhizine shows very clearly the method by which the very numerous bridges connecting the internal hyphae of the mature rhizine arise (Figs. 9 to 12). A single hypha at the tip of the rhizine outgrows its neighbours (Fig. 12). After it has grown a short distance another hypha grows up beside it, the tip of which turns in at right angles, and grows towards the first hypha and fuses with it. Its apical growth is thereby terminated, but it soon recovers and continues to grow in the original direction, i. e. at right angles to the old hyphal tip. By the fusion just described a bridge is formed between two neighbouring hyphae, across the middle of which a transverse wall generally grows. Walls are also ultimately formed in both of the hyphae concerned below and above the fusion bridge. These strictly right-angle fusions are very numerous near the tip of the young rhizine, and the hyphae thus become firmly united in all directions at a very early stage of growth (Fig. 10). Arthur Meyer describes and figures such completed fusions between the rhizinal hyphae in *P. canina* (17, p. 162), and also similar completed apical fusions among the epicortical hairs, but he does not show the method of their origin and the subsequent growth of the hyphae concerned (id., p. 162). He is surprised to find so many fusions between the hyphae of the rhizine, and he speaks of the apical hyphae of the rhizines being thinner and freer from one another, but yet exhibiting H-fusions. From this it is clear that he did not understand the manner of their origin. He appears to consider that the fusions arise among the older longitudinal hyphae of the rhizine as lateral outgrowths some distance away from the apex of the rhizine. But my evidence points to the fact that practically all the fusions which are found in the longitudinal hyphae of the rhizine are formed at the apex of the rhizines, and especially at the very tips of the separate hyphae. Right-angle fusions are described and figured by Meyer as already completed for *Hypomyces* (id., p. 162, Figs. 32 and 34). Other authors refer to the fusions observable in the rhizines of *Peltigera*. Lundegårdh has briefly summarized the occurrence of fusions between hyphae among fungi, from which it appears that very little is known about them (16, pp. 139, 140).

This peculiar form of apical fusion which results in an H-connexion between neighbouring hyphae is met with in all the species of *Peltigera* which I have examined and when the hyphae are growing freely. They occur

in the developing hypothallus, among the medullary hyphae when the latter are exposed by a cut or other injury, among the young epicortical hairs, and in the young embryonic protothallus generally. But the course of their development can be best seen in the undifferentiated tips of young rhizines.

The outer hyphae just behind the apex of the young rhizine of *P. canina* gradually become differentiated into the loose outer tissue which is continuous with the hypothallus of the main portion of the metathallus (Fig. 10). In this part of the rhizine the hyphae run in practically all directions, whereas the inner hyphae of the rhizine all run longitudinally. The structure of the mature rhizine shows that the inner longitudinal hyphae are large at the centre and get smaller in diameter towards the periphery, where they pass rather abruptly into the loose outer hyphae which run in all directions. The inner hyphae are all extensively connected by the fusion bridges already referred to, which run at right angles to the longitudinal axis of the rhizine (Figs. 13 to 15).

The central hyphae of an ordinary rhizine are up to  $8\mu$  in diameter when mounted in a watery medium like glycerine jelly, while the walls are about  $2\mu$  thick. Mounted in a medium which demands previous dehydration, the measurements are about  $7\mu$  and  $4\mu$  respectively. Towards the periphery those hyphae which are still running in a longitudinal direction have a diameter of  $4\mu$  in whatever medium they are mounted, but the walls are  $1\mu$  thick in a watery medium, and only  $0.5\mu$  thick in one requiring dehydration. The outer loose hyphae which form the hypothallus-like covering measure 4 to  $6\mu$  in diameter and have walls of a thickness of about  $0.5\mu$  in whatever medium they are mounted. These figures show the extent to which the walls of the central hyphae can absorb water. The measurements given by Sievers (30, p. 18) for *P. canina* are throughout lower than those recorded here, especially with regard to the thickness of the walls. This may be due to individual differences or to the age of the rhizines.

The short bridges connecting the central hyphae are about  $4\mu$  in length, and generally slightly narrower than the two hyphae they connect. Many of the central hyphae seem to be cemented together by their walls without the contents actually anastomosing. There are many interhyphal air-spaces in the rhizines, and these, owing to the close union of the surrounding hyphae, often take the form of longitudinally running hollow tubes. We can distinguish three transverse regions in the whole length of a mature rhizine of *P. praetextata*. There is first the undifferentiated apex which may still be actively growing. This is followed by the typical well-differentiated portion of the rhizine which makes up the greater part of the whole organ. This consists of the longitudinally running inner hyphae, surrounded by the irregularly running hyphae of the outer tissue. Finally



there follows the region of attachment of the rhizine to the main thallus, and especially to the veins.

Many of the hyphae enter the rhizine obliquely by crossing over from one side to the other as they leave the region of attachment, although the whole rhizine is at right angles to the direction of the veins (Figs. 1 and 2).

The general appearance of the whole rhizine of *P. canina* varies to a certain extent, especially at its tip (Figs. 6 and 7). When touching some solid substratum, as a moss leaf or another old *Peltigera* thallus, or in artificial culture a glass slide, &c., the rhizine may break up into a number of thin strands, each consisting of bundles of closely united hyphae, characterized by numerous bridge-fusions (Fig. 7). When growing on leaf-mould or soil that can easily be penetrated, the tip of the rhizine may break up in a brush-like fashion (Fig. 6). Numerous separate hyphae pass into the soil almost independently of one another and fusions are absent or very rare. Generally the part of the rhizine which lies between the point of attachment to the main thallus and the substratum has numerous short hyphae which project rather in the manner of root-hairs (Figs. 6 and 7).

The relatively great length of the rhizines of *P. canina* when compared with the thickness of the metathallus is very striking. The rhizine may be as thick as the mature thallus, but about eight to ten times as long (Fig. 6).

In forma *spongiosa* of *P. canina* the rhizines are more loosely built and branched (Fig. 3). From an early stage of growth numerous hyphae are given off from all sides of the rhizine which point downwards at an angle of  $45^\circ$  with the longitudinal axis of the rhizine from which they arise. The rhizines of this form arise very closely together, and often become united by a single hypha or strands of hyphae, so that in the end the whole under side of the thallus forms what Tuckerman calls a nap (36, p. 109). Separate hyphae from the rhizines in this form and in other species may completely surround and cover moss leaves and stems and old *Peltigera* thalli. These hyphae are rarely connected to one another by bridge fusions, and do not seem to penetrate into any living cells. If they kill the plants thus attacked, they do so by keeping out the light and possibly by depriving the plants of food material. M. and Mme Moreau describe two kinds of rhizines for *P. canina*. One of these they call bundles of hyphae, which are figured by them as forming their subterranean mycelium. I mentioned in a former paper that I did not quite understand the figures in question (8, p. 16). To me they appear to be at least incomplete. The same authors also describe and figure another type of rhizine to which they apply the term 'crampon'. I venture to think that in this case they were dealing with the dead leaves of some moss which are frequently found in very close association with the loose hyphae of the

hypothallus of any species of *Peltigera*. Such a moss leaf or 'crampon' is shown in the figure of *P. canina*, f. *spongiosa* (Fig. 3).

In *P. horizontalis* the big black rhizines are much branched, but their internal structure is the same as in *P. canina* (Figs. 4, 6, and 8). They often arise in rows of equal age, running concentrically with the growing margin of the thallus. When mature they may attain a length of 3.5 mm. A short narrow neck here connects the rhizine to the metathallus. The rhizine is made up of numerous compound strands of hyphae, all united at the point of attachment to the metathallus, but spreading out towards the substratum. These strands branch and anastomose freely, as do the separate hyphae, by the usual methods. The tips of the hyphae seem to spread out wherever they come into contact with a flat object. The walls of the hyphae then seem to become firmly cemented to the substratum. The apical hyphae often begin to spread out like root-hairs in a damp atmosphere before they actually reach the substratum or light, whilst the loose hyphae are united by the usual bridge fusions. Over old moss leaves the hyphae of the rhizines spread out, and remain almost free of one another except for the usual bridge fusions. There is thus created a network which becomes closely cemented to the flat substratum; very occasionally hyphae will be found to have penetrated into the substratum (Fig. 11). Here, as in all other species, all the rhizines are primary in rank. No secondary rhizines are formed between the older ones.

Rosendahl (25, p. 411) considers the origin of the rhizines, or rhizoids as he calls them, in the case of *Parmelia aspidota* to be due to a contact stimulus. This, however, is certainly not so in the case of the species of *Peltigera*. Here the rhizines may grow out even to a length of several mm. before coming into contact with any substratum. This I have observed in *P. canina*, *P. praetextata*, and *P. horizontalis*.

The function of the rhizines is clearly twofold. They attach the plant to the substratum and are thus organs of attachment, but they also raise up the horizontal thallus from the substratum in order to prevent general too close contact with the latter, because this would interfere with the free admission of air to the under side, and thus with the supply of carbon dioxide gas to the gonidia. They absorb water and are thus organs of absorption. In these two respects they correspond in function to the roots of green vascular land-plants.

In the literature there are numerous references to the function of the rhizines. In his text-book Schneider describes the rhizoids as guys fastening the thallus to the substratum (26, p. 83), and he refers to the 'soluble food taken up by the rhizoids' (id., p. 47). Zukal considers the rhizines of *Peltigera* as having tensile strength (38, p. 1393), and of being of importance for the supply of water to the lichen (id., p. 1339). The water, he says, rises in the rhizines solely between the hyphae by capillarity (id.,

p. 1340). This statement is quoted by Birger Nilson (20, p. 7) in order to combat Reinke's view, which states in a general way that the water is transported in the hyphae themselves. Miss Smith (31, p. 92), speaking of the rhizines as rhizoids, or rootlets, follows Nilson, and says that 'these are mainly for the purpose of attachment and have little significance as organs of absorption'.

Sievers, after repeating some experiments first attempted by Zukal, comes to the conclusion that when water rises in the rhizines it does so not only intercellularly (30, p. 19), but also within the walls and the lumina of the hyphae. I have carried out numerous experiments with eosin in order to determine the exact course of the water-current, mainly in the case of *P. horizontalis*. When the tip of the rhizine is dipped into a watery solution of eosin, the latter rises almost instantaneously to the top of the rhizine, and at once hypha-wall and lumen draw on this supply of water held by capillarity in the intercellular spaces of the rhizine. This is the first and most readily obtained store of water, which is drawn off as rapidly as possible by the walls and lumina of the hyphae, and passed on to the conducting tissues of the horizontal thallus and along the hyphae of the latter at an observed rate of about 1 mm. in 2 minutes. In this way the water-drawing mechanism of the rhizine becomes ready to raise more water in a short time. The structure of the rhizine is admirably adapted for drawing up water rapidly by capillarity. The vessel-like hollow passages running longitudinally have already been referred to. Sections of the central hyphae cut immediately after dipping a rhizine into the red solution and blotting the material to get rid of all intercellular liquid show red cell-walls and lumina. If these are then mounted in pure water the colour in the walls soon becomes weaker. As soon as the water has been brought up to the veins, it seems to be taken along inside the hyphal walls and lumina, between which there are very few air-spaces. As practically no water is absorbed by the upper side of *Peltigera*, the rhizines are normally the only water-absorbing organs, and this accounts for their great development. We must realize that our lichens are xerophytes, not only in the general sense as members of Church's xerophyton, but in a special sense and to a much greater degree. They require water, but the depth of substratum which can supply this is very small. Further, lichens do not possess suberized walls or layers of cork cells, which form a most important part of the equipment of the vascular xerophyte. These are all reasons why the water-absorbing organs should be well developed and the transpiring surface should be as small as possible. Of course the demands for light also play an important part in shaping the form of the whole plant.

The rhizines function as organs of support to the horizontal thallus and of attachment to the substratum. The great mechanical strength of the

rhizine is due to their extensive anastomosing of its hyphae. If it were possible to pull at any one of the hyphae projecting from the growing-point of a young rhizine, it would be found that it would yield only slightly, as at even an early stage it is firmly connected either directly or indirectly with practically every other hypha at about its own level. The right-angle fusions act as struts. The rhizines possess considerable tensile strength, the strongest hyphae being found near the centre of the rhizine, where they are more firmly bound together than at the periphery. This is what one would expect to find from what is known of similar organs among vascular plants. The oblique course of the hyphae at the upper end of the rhizines must add considerably to their power of withstanding lateral tension. We see, then, that the rhizine of *Peltigera* is admirably adapted to carry out the duties not merely of a colourless fungus, but of a set of organs forming part of a green lichen-organism.

It is permissible here to call attention to the similarity in the behaviour of the ascus-forming hypha and of the growing *Peltigera* hypha as seen at the tip of a rhizine. The penultimate and not the apical cell of a fertile hypha forms the ascus owing to the hook formed at its tip. A similar hook is formed in the young vegetative hyphae and the penultimate cytoplasm, if not the cell, grows on. In both cases the actual tip undergoes fusion.

#### *The Structure and Development of the Isidia.*

Isidia are small outgrowths from the upper surface of the mature lichen-thallus of crustaceous and foliose lichens. Du Rietz has classified the various types of isidia met with (24, p. 371), and his definition of an isidium as an organ produced by a simple protrusion of the cortex (Ausstülpung, id., p. 381) can be applied to such cylindrical isidia as we get in *Parmelia saxatilis*, *Pertusaria coccodes*, *Umbilicaria pustulata*, &c., but not to the isidia which are found on the thallus of *Peltigera praetextata* and *P. lepidophora*, in which the cortex is broken through completely. He introduces the term isidangium, suggested in a lecture by Sernander (id., p. 379), for a group or sorus of isidia, referring specially to *P. praetextata* and *P. lepidophora* (id., p. 382). The isidia of *P. lepidophora* have been described by Bitter, who considered them as representing a new type of cephalodium (3, p. 251), and by Linkola, whose observations point to their being true isidia (14, p. 52). The organs in question differ from the isidia met with in *P. praetextata* in certain external features, in which they closely resemble the cephalodia of *Peltidea aphthosa*. According to Linkola the gonidia are not of extraneous origin, as Bitter maintains, but come from within the lichen. Sections I have cut of *P. lepidophora* (Arn. lich., No. 1469) fully confirm the view of Linkola that the gonidia of the isidia come from the ordinary gonidial layer of the metathallus.

The isidia of *P. praetextata* are small leaf-like organs which are found

either on the mature portions of the thallus, i. e. the metathallus, or at the margin, i. e. the protothallus (Pl. XXXI, Fig. 37). It is with the former that the following observations are mainly concerned. They appear to arise on the sides of cracks formed in the cortex of the metathallus. Cracks occur in the metathallus of several species of *Peltigera* and they may be several millimetres in length (Pl. XXIX, Figs. 18, 21 to 27). They are probably due to the continual rolling up of the thallus when it is dry, and its unrolling when it becomes moistened again. If this folding takes place frequently and along the same line a crack will occur in that tissue which is oldest and has most completely lost the power of flexibility. That is apparently what happens. The cortex, which is a tissue of thick-walled cells firmly cemented together, breaks, but the inner tissues, being more loosely interwoven, and still having to a considerable extent the power to grow out, whether injured or not, remain unbroken. The usual direction of the cracks seems to point to this explanation as being the correct one. Although cracks may be found which run in any direction and which even cross one another, it is possible that the first cracks are formed in the way suggested above, but that later, owing to old age or to cracks once formed continuing in other directions than the original one, the cortex may break open in any direction. Strato is of the opinion that cracks are due entirely to the drying up of the thallus (34, p. 24). I do not think that they are formed by the cortex being unable to keep pace with the growth of the medullary tissues. When an artificial incision is made the wound does not gape. At the same time, subsequent growth of the medullary hyphae would tend to separate the two edges of the wound. The possible origin of the break-through by the activity of internal hyphae and from within will be discussed later in this paper. Apart from *P. praetextata* cracks are commonly found in *P. polydactyla*, *P. canina*, and *P. horizontalis*. But in the last three cases the wounds are healed over without forming any isidia. The frequent cracks found in *P. polydactyla* (Figs. 18 and 21 to 26) generally run concentrically to the margin. They are healed without giving rise to isidia and they never seem to extend to any great depth. The cortex is broken through first and the gonidial layer is thus laid bare. The crack runs right across the cells as a rule (Fig. 22) and breaks through the transverse walls of the cortical hyphae. Even in its very earliest stages such a crack presents a threat of desiccation to the gonidia. The intergonidial hyphae, without growing out, seem at first, if suitably placed, to form a protective layer for the exposed gonidia. Only in very few cases have I found dead gonidia in connexion with a crack. The hyphae of the medulla immediately below the gonidial layer, which need not necessarily be injured by the formation of a crack, send up branches into the wound and ultimately form a new cortex, the discontinuity of which with the old primary cortex can generally be well made out, even when the wound has been healed over completely. The scar of

the crack seems never to disappear. Gonidia are at first absent from just below a crack. This also seems to point to an extension by growth on the part of the medullary hyphae after the formation of the crack. At first the gonidia seem to take no part in this extension, but ultimately they apparently find their way into the regions below the crack. In cases where the cortex has been completely removed, by the action of some small animal for example, a new cortex can be developed from the intergonidial hyphae and to a greater extent from the growing out of the medullary hyphae. Thus the wound is healed and a new cortex formed (Figs. 25 and 26).

I have often observed the healing of cracks in *P. canina*, f. *spongiosa* (Pls. XXVIII and XXIX, Figs. 3 and 27). The medullary hyphae grow out into the wound and even cross over to the cortex on the opposite side of the wound. Here, again, no isidia are formed, once the hyphae from the medulla have healed up the crack and the lichen has regained control over the transpiration current.

Cracks that have completely healed over have been frequently seen by me in the case of *P. horizontalis*. Isidia are not formed here either. I think there is no doubt that the cracks in the case of these three species which do not produce isidia are due entirely to external agencies. They apparently contain no tissue which can be stimulated by internal or external stimulus to break through the cortex.

We owe to Strato an account of the occurrence of cracks and isidia in an undulate form of *Peltigera canina* (34, pp. 22 to 24), but I think that there is no doubt that his observations were really concerned with *P. praetextata*, as has been pointed out by other lichenologists (Linkola, 15, p. 89; Tobler, 35, p. 26).

Very generally isidia occur in large numbers only on the metathallus of *P. praetextata*. They originate below the level of the cortex, and this must be broken through before they can develop. It is not always easy to see in what way the cortex has been broken through. A crack in the upper cortex may arise in the manner described above. In that case it is more or less evidently due to an injury, caused by some external mechanical effect. A narrow crack thus formed gradually widens out to form a bigger gap as the underlying tissues increase in extent. I have not, so far, found in any *Peltigera* species in nature an obviously newly formed crack, or even one recently healed over, which could be definitely interpreted as being due to some external mechanical effect.

I have carried out numerous experiments in cultures with material in Petri dishes. Small square pieces have been kept under moist conditions for a year. Others have merely had incisions made to varying depths. Typical isidia are not developed as a rule, least of all when the margin or edge has been completely removed. Small round bodies are formed which for convenience I have called 'regeneration nodules'. These differ from

normal isidia by being more loosely attached to the thallus, and from soredia by having a fairly smooth outer fungal envelope. Linkola has already pointed out the differences of these regenerative 'isidia' from soredia (15, p. 70). They seem to represent an abnormal state and occur in several if not all species of *Peltigera* I have experimented with. They do not seem to occur in specimens growing out in the open. I hope to deal with them in a separate paper, which will be devoted especially to regeneration phenomena. Strato carried out experiments with injured thalli of *Peltigera canina* in order to observe the progress of regeneration (34, p. 31). I wish here only to call attention to his view that it is the gonidia which initiate the formation of the isidia once the cortex has been ruptured (id., p. 24).

Linkola kept some plants of *Peltigera praetextata* under observation in the open for nearly two years (15, p. 71). He found that isidia had been formed at the end of that period only where two years previously artificial incisions had been made. All these experiments were really carried out under artificial conditions and do not necessarily explain what happens normally under natural conditions.

There is evidence that, in *P. praetextata* at any rate, hyphae may arise from the medulla before the cortex has been broken through and may push their way in between the cells of the latter, thus making a passage through the upper cortex (Pls. XXX and XXXI, Figs. 29, 30, and 48). These hyphae are in appearance like neither the ordinary cortical hyphae nor those of the medulla. They are smaller in diameter than the former, and they may already at an early stage show the darkening of the walls and the close lateral branching characteristic of the hyphae found at a later stage in the open cracks. These hyphae can be found making their way through the cortex and at the same time to be closely surrounding the separate groups of gonidia as if in preparation for their becoming exposed. This kind of activity among the medullary hyphae may extend from a point where isidia have already grown up well above the level of the cortex to such points under the cortex where the latter is still in a normal and undisturbed condition. It may, however, also be observed in portions which are still completely covered by the cortex. It has been observed both in fresh material sent from Finland and in fresh material collected in England, and in neither case could I detect any external influence which might have initiated it.

Macroscopic circumstantial evidence in favour of the view that the isidia do not necessarily arise in cracks is afforded by the observation of small groups of isidia or isidangia on the surface of the metathallus, apparently unconnected with any crack. It is, however, just possible that they have grown up through a small hole where the cortex has been pierced by some insect or other animal. In a few sections small groups of *Nostoc* cells were noticed on the outside of the cortex, but I do not consider that these can have been the cause of the fungal activity just mentioned, which

begins low down among the medullary hyphae (Figs. 29 and 35). There is no evidence that such algae are, even later on, embodied in the gonidial layer of the isidia. Strato, however, placed some loose masses of *Nostoc* cells, i. e. gonidia, on the uninjured portions of a thallus and found that after a few weeks isidia had formed, as the cortical hyphae had grown up round the algae (34, p. 29, Fig. 10). His figures illustrating this point show a structure very much like the regeneration nodules I have referred to. In any case, in my experience, it takes more than a few weeks for even the youngest stages of the isidia to develop, so that I am sure further experiments are necessary to settle this point.

Linkola has given a brief account of the origin of isidia in *P. praetextata*. He considers that they arise away from the margin only as the result of wounds (15, pp. 70 to 71) caused by external mechanical means. Whether that is so or not in *P. praetextata*, there are clearly among the medullary hyphae some specialized elements the function of which is to take an important part in the formation of the isidia, and of which I can find no trace in any species of *Peltigera* in which no isidia are formed (Pl. XXXI, Fig. 48).

In places where a crack is already giving rise to isidia, medullary activity is often exhibited some little distance from the actual crack, and well below the cortex even before there is a clear direct way to the outside, at that particular spot at any rate. But it is just possible that these hyphae may ultimately reach the surface by the widening out of the original crack and not by making a new crack for themselves. In any case they may be considered to represent a spreading of that activity which resulted in the formation of the isidia in the first instance.

The cells resulting from the growth of the medullary hyphae into the interrupted cortex are in the first stages small and short when in immediate touch with the gonidial layer and longer when growing freely in the still narrow gap of the crack or when growing towards it (Pl. XXX, Figs. 28, 31, and 35). Even these cells show the very characteristic method of growth referred to above of sending out branches at short distances without at first forming the corresponding transverse walls (Figs. 29 and 31). This results in the formation of cells with slightly wavy outlines as the hyphae become closely cemented together. Strato was the first to refer casually to these cells (34, pp. 23 and 36, Fig. 13). Wavy outlines of surface cells are figured by de Bary in the case of the cortical cells of the sclerotium of *Typhula gyrans* (1, p. 33, Fig. 15, c) which represent the peripheral segments of medullary hyphae. The cells covering the youngest isidia arise from the medullary hyphae only, and if examined in surface view exhibit this form of growth very clearly. The cells form an uninterrupted outer wall and practically dovetail into one another (Figs. 32 and 33).

By this time the gonidia are taking an active part in the increase in size of the isidia. They are growing vigorously, as is shown by their size being



smaller than that of those in the normal vegetative condition of the mature thallus (Figs. 32, 34, and 36). At this stage the close co-operation between algae and fungus is very evident. The former are pushing on and the latter is doing its part of the work by keeping the gonidia well covered. Strato was inclined to make the gonidia the prime movers in the formation of the isidia once the cortex has been ruptured. He imagined that the gonidia exposed by the cracking of the cortex push their way up as the result of their loose covering and the influence of moisture (34, p. 24). This is not in accord with all my observations. These all point to the fact that the fungal constituent of the lichen grows out to protect and cover the gonidia to which injury and desiccation are threatened by exposure in the crack. If the break-through in the cortex is not due to any external mechanical stimulus, but only to internal activity, then it is certainly the fungus which breaks through the cortex in the first place. Though in this way the fungus may actually be ahead of the algae at one time, it will never go so far ahead as to outgrow the gonidia so that these cannot follow and keep up with it. These organisms are working together and influencing each other the whole time. The figures given by Strato (id., Figs. 8 and 9) do not represent normal isidia either in their earlier or later stages. At the same time his brief account of the earliest stages of the origin of the isidia is correct (id., p. 25). Tobler (35, p. 25) agrees with Linkola and Strato (34, p. 26) as to the origin of the isidia.

The actual break in the cortex is never healed by the cortex itself, as the cortical cells have lost the power of regenerative growth. The abrupt ending of the old cortex can be seen (Figs. 32 and 34 to 36) in all cross-sections of cracks, though it may become less obvious in old specimens. The activity of the medullary hyphae at first does not go beyond the inner limits of the old torn cortex. Not until the crack is practically healed over from cortex to cortex by the activity of the medullary hyphae do the gonidia really show any signs of steady growth (Fig. 34). The whole of the isidial activity of the lichen *P. praetextata* originates below the old ruptured cortex. The edges of this cortex are later on pushed back and practically always remain distinctly visible.

At first the gonidia seem to spread in all directions without arranging themselves in any definite order inside the isidium (Figs. 33 and 34). This remains so as long as the whole isidium has not reached the level of the cortex. The young isidium ceases to be spherical in form and becomes drawn out when it comes to the level of the upper surface of the thallus. A rearrangement of the gonidia can now be observed from which the future position of the upper and under sides of the mature isidium respectively can already be made out (Fig. 32). The gonidia finally lie in rows running roughly perpendicular to the surface of the future upper side of the isidium (Fig. 36).

The structure of the fungal tissue of the isidium exhibits an important differentiation. It may again be pointed out that there is a continuous covering layer across the gap of the crack from old cortex to old cortex, the abrupt endings of which are still clearly seen (Fig. 36). The already distinct stalk of the isidium is made up of very large squat cells on the outside, many of which can be seen to be the end cells of very much narrower medullary hyphae (Figs. 32 and 36). Inside the stalk are the long-drawn-out hyphal cells connected to the ordinary hyphae of the medulla on the one hand and the inner tissues of the young isidium on the other. Between them are the gonidia, which are here much elongated and few in number. There are plenty of air-spaces between these hyphae, whose function is certainly that of conduction. Branches of the same medullary hyphae form at first the covering cells of the isidium proper (Figs. 33 and 34). These are very small and thick-walled, and in a surface view are seen to have wavy outlines (Fig. 33). These cells, which become closely interwoven and firmly cemented together, represent the apical portions of the medullary hyphae of the main thallus. The wavy outlines of the cells are due to the hyphal ends bending at right angles and then continuing to grow out afresh in the old original direction, as we noticed in the case of the apical hyphae at the tip of the rhizines (Fig. 33). Attention has been called to the characteristic behaviour of these hyphae, which ultimately give rise to the isidia. The young isidium is covered on the outside with a single firm and continuous layer of thick-walled and much-darkened cells which all have a wavy outline (Figs. 32 and 36).

The cells of the under side, which are gradually becoming differentiated, correspond to the hypothallial cells of the metathallus, the differentiation of which begins in the protothallial region. They are not primarily connected with one another except in groups of possibly two or three. They arise from the inner medullary cells of the isidium just as the hypothallus is connected with the undifferentiated medullary hyphae of the protothallus (Pl. XXXI, Fig. 47). They are provided with lighter-coloured walls and they vary considerably in size, though they mostly exhibit already wavy outlines. It is in this region that active new hyphae are pushing their way in to increase the area of the under side (Figs. 39 to 46, and 48 to 51).

The further growth of the isidia seems to take the following course. At first growth is most active on the future under side, and it is in this way that the gonidia are gradually pushed round and into the position they will occupy in the mature isidium. When once well established the method of growth already described for the prothallus can be made out in the isidium. The medullary hyphae and their branches give rise to the outer cells of the under side and to the dark cells of the front and upper side, all of which possess wavy outlines. Inside and just below the latter are the more delicate cells which later on are going to emerge

from beneath these thick-walled cells of the upper side as the normal cortex of the mature isidium. The tissues of the mature isidium thus show a firm many-layered cortex on the upper side (Figs. 38 and 49). Below this cortex are the gonidia, and below these, though only slightly differentiated, the loosely interwoven medullary hyphae. The under side is always one layer thick only. I do not agree with Strato, who referred to the structure of the isidia in an undulate form of *P. canina* (already stated to be in reality *P. praetextata*) as being much more irregular than the ordinary thallus (34, p. 23). It is as regular, if not more so, and exhibits a very high degree of physiological differentiation (Fig. 49).

The cortex of the upper side of the mature isidium is of the same appearance as the cortex of a young portion of the ordinary metathallus. At the growing margin, and also at a lateral margin which has ceased growing and should then perhaps better be called an edge, we find the normal isidial cortex covered by a layer of small cells (Fig. 38). These are thick-walled, dark in colour, and have wavy outlines. It is between these that the normal cortical cells gradually emerge which are cylindrical in surface view and do not show any wavy outlines (Figs. 39 to 41). The thick-walled covering cells correspond to the hair-like felt of loose hyphae covering and protecting the young tissues of the ordinary protothallus. Instead of rising up, spreading out, and branching freely, they remain closely applied to the apices of the developing cortical cells. On the upper side they may pass gradually into the normal cortex of the mature isidium (Fig. 40) or run into short hyphae between which the cortical cells may be seen to emerge (Fig. 39). In surface view they stand out very clearly against the normal and permanent cells of the cortex.

A surface view of the continuous covering of the under side shows various points of interest in the case of an older isidium. Towards the base or stalk the cells are larger and fit closely together, and show wavy outlines (Fig. 44), while nearer to the margin of the isidium they are smaller (Fig. 42). This does not mean that the large cells of the stalk end are in due course going to divide and become smaller. It is in the area lying between those big cells of the stalk and the smaller ones of the margin that new hyphae enter the under side (Fig. 47). On the under side the layer of cells can be seen to be interrupted by occasional openings or pores (Figs. 43, 45, 46, 49, and 50). Although the ends of the hyphae which have built up the under side by forming the short branches referred to above become closely cemented together they nevertheless leave well-defined but minute openings or pores of a regular oval shape. These openings are surrounded by two or rarely more cells (Figs. 43, 45, 46, and 50). They can be seen, though not always easily, in the surface view of sections cut parallel to the surface or by looking at uncut material in surface view (Fig. 50). Sections cut perpendicularly to the surface do not often clearly show these openings, as even

with the most careful preparation the sections are apt to tear at a place where the cortical continuity is interrupted by a pore (Figs. 38 and 49). Thus the neighbouring cells of a pore would often be indistinguishable as such. The walls of the cells bordering on a pore are often like those of guard-cells in shape, and even seem to be unequally thickened. It is impossible, owing to the minuteness of the objects concerned, to see whether the bordering cells change their shape under varying conditions of moisture and light. There is no doubt that they function as organs for a gaseous exchange between the air-spaces inside and the air outside the isidium.

As regards both development and structure the pores in question differ markedly from the stomata of mosses and vascular plants. The two guard-cells of the latter are derived from one stoma mother-cell. In the case of *P. praetextata* the two or more cells surrounding the opening may have originated from several different hyphae. The surrounding cells in one particular case of *P. praetextata* measured 4 to 5  $\mu$  across, the actual opening measuring  $8 \times 11 \mu$ . These figures correspond, as far as the width of the pore is concerned, to the size of the passage of an open stoma in *Amaryllis formosissima* (Haberlandt, 11, p. 449), but the guard-cells of the latter are bigger than the cells surrounding the pores in *P. praetextata*.

It is not difficult to see how these openings are formed when we recall the method by which fusions take place between neighbouring hyphae at the tips of the rhizines. The growth of the medullary hyphae of the isidia, while forming the lower cortex, represents the same process. If I am justified in assuming that these openings in *P. praetextata* are of importance for gaseous exchange, especially in connexion with the photosynthetic process, they represent good evidence in favour of the view that in the lichen organism fungus and algae work together as one organism and not as two. During the process of development the hyphae forming the under side of an isidium grow up close together and undergo fusions like those at the tips of the rhizines. Thus gaps are left between the hyphae just at the tips of the rhizinal hyphae. The threat of desiccation to the gonidia acts through the latter on the fungus and the gaps then tend to close up. Shortage in the supply of the necessary carbon dioxide would tend to keep them open. Fungus and algae work together and function together as one organism as completely as green tissue and non-green tissue do in an ordinary green vascular land-plant.

Without going back to any original source, I can briefly refer to Stiles and his summary of photosynthesis (33). The gaseous exchange in connexion with this process takes place almost exclusively through the stomata in the case of the higher green land-plant. Again, the carbon dioxide thus absorbed is used for the formation of starch only in the immediate neighbourhood of the point of absorption (id., p. 63). These are facts which have been demonstrated in the case of higher plants, and they can be taken as

evidence in favour of the view that the pores on the under side of the isidia of *P. praetextata* act as organs for admitting carbon dioxide for purposes of photosynthesis. The only other passage by which air could be admitted to the isidial gonidia is by one of the cyphellae on the under side of the main metathallus. From here it would pass through the air-spaces of the medulla and of the isidial stalk and thus finally reach the gonidia in the isidium. This would mean an unusually long distance for the carbon dioxide to travel in the plant. The isidia do not possess any cuticle, so transpiration takes place either by the cortex alone or by the cortex and the pores. Oxygen will enter by these pores even though all the oxygen an isidium requires would be available through the air-spaces of the stalk. All this goes to show that the pores on the under side of the isidia are of importance for the photosynthetic process.

According to Haberlandt loss of photosynthetic function has in the case of sporogonium of *Sphagnum* (11, pp. 21 and 475) led to the reduction of the stomata. Guard-cells are here still initiated, but pore and internal air-chamber remain undeveloped. This emphasizes the intimate relationship existing between pore and photosynthesis. At the same time stomata are found functioning in certain actively growing organs in the entire absence of chlorophyll, if only in the interests of respiration (id., p. 475).

So-called breathing pores or 'Atemporen' are already known in lichens. But these are pores of the coarsest structure. They include the cyphellae of the Stictaceae, the breathing pores of the Parmelias (Rosen-dahl, 25, p. 412), or the 'Atemporen' of *Ramalina* (Darbishire, 7, p. 7), and of course the cyphellae described for the metathallus of *Peltigera* in this paper. I have no doubt that most of these openings answer the purpose, at any rate, of complete gaseous exchange. In *Ramalina fraxinea* the green gonidia are seen to be most crowded at the inside of the funnel leading to the external pore.

The structure of the isidium as a whole is extraordinarily like the leaf of an ordinary green land-plant (Fig. 49). There is the many-layered upper cortex covering and protecting the gonidia, but not provided with pores. Then follows the gonidial layer with the separate cells arranged in palisade-like rows and the loose tissue of the medullary hyphae forming a kind of spongy mesophyll which is covered by a thin lower cortex, one cell deep, and provided with occasional open pores. There are the cortical cells of the lower side exhibiting the wavy outlines, and lastly the large hyphae leading in by the stalk and bringing in water and solutes and probably carrying away the products of photosynthesis. The isidium as a whole is sensitive to light, and in the end it takes up the best light position. There are no strengthening veins such as occur in the higher plants, probably because the isidium is too small and does not require any extensive mechanical strengthening system. Instead, the upper and under sides are

held firmly together by hyphae connecting the upper to the under cortex. There is no cuticle, so that transpiration probably takes place through the cells of the upper cortex, just as in the metathallus of the main body of the lichen.

The isidium is at first a simple flattened structure, but gradually, as the margin grows out unevenly, it becomes branched (Fig. 37). It is not difficult to see then at which points growth is most active. At such points the gonidia are seen to be most densely crowded. This should, however, not be interpreted to mean that the gonidia are the prime movers in the growth of an isidial branch. In a large isidium there may be something like thirty to forty points at which such growth is proceeding. At these points new hyphae from the inside of the isidium are coming to the surface near the margin to make new cortical cells on the under side.

At first only a single row of isidia is produced by a crack, but very soon the isidia grow out laterally to form the branches already mentioned or quite new isidia arise at their bases. Small groups (or isidangia) of crowded isidia can be formed, of which all members exhibit the same orientation of tissues and of the upper and under sides.

It is not quite easy to determine exactly the function of the isidia. I think the primary function is that of increasing the surface of the thallus for the purpose of increased photosynthesis and water transpiration as an aid to this. This is the view I expressed in a paper published in 1897, in discussing the isidia of *Pertusaria coccodes* (6, p. 603) and *P. coronata* (id., p. 604). The isidia of these lichens differ from those of *Peltigera praetextata*. The latter break through the cortex of the metathallus and subsequently develop their own cortex. The former have a cortex which is formed by interpolating new cortical hyphae between those of the original old cortex. Owing to the fact that in these the gonidia are crowded at the apex, my description of them is quoted by Kajanus (13, p. 36) as evidence for a view strongly held by him (id., p. 35) that the gonidia under the influence of moist conditions are the prime movers in initiating the formation of isidia.

The isidia of *P. praetextata* are very firmly fixed to the main metathallus. Their removal for reproductive purposes would therefore mean the creation of an open wound of some size, not only on the main thallus but also at the base of the isidium. This would be of a quite different nature from that formed by a crack. The latter is narrow and merely exposes the gonidial layer to a very limited extent. The former would go right across the gonidial layer of the isidial stalk. It would also require some force to remove an isidium. So that I do not think that the isidia are primarily reproductive organs, at any rate in the case of *P. praetextata*.

I have separated several isidia and kept them under observation in the moist chamber of a Petri dish for thirty-five days, after which some had certainly sent out medullary hyphae to a distance of 130  $\mu$ , which exhibited

the characteristic bridge fusions at their apices already noticed in the case of the rhizines. But the gonidia soon lost their colour. I have no doubt that isidia could settle down if separated from their parent thallus and placed in suitable conditions on an appropriate substratum. But they cannot for that reason be considered to be reproductive organs any more than a cutting of a willow tree could be considered as such even if when successfully planted it reproduced the parent plant from which it was taken. I have not, however, so far been able to obtain an undoubted independent *Peltigera* thallus from an isidium separated from its parent plant. Strato was able to do so only to a limited extent, as he did not succeed in keeping his isidium growing for more than two months (34, p. 30). So nothing definite can be gathered from this experiment. Linkola also suggests that the isidia might be vegetative reproductive organs (15, p. 74). I must admit that I have found in nature small thalli apparently of *P. praetextata* which might have represented isidia which had become detached and had settled down possibly after the parent had died. Reinke figures some such small thalli (22, p. 455). But the question must still be left an open one.

I would like here to refer again to *P. lepidophora*. When in this species the gonidia pass from the gonidial layer and reach the level of the cortex they are covered by a dark layer of thick-walled cells, just as is the case with *P. praetextata* and its young isidia. This layer is well shown in Linkola's figures (14, p. 52, Pl. II, Figs. 1 to 4). These cells exhibit the wavy outlines characteristic of the corresponding cells of *P. praetextata* from the very beginning. But I was unable to determine how they originated. Unfortunately I had no fresh material at my disposal. The structure of the mature isidium of *P. lepidophora* is very similar to that of *P. praetextata*. The upper cortex generally has fewer cells in each row. These cells are round though cylindrical in section and bigger, and often only one such cell separates the gonidial mucilage from the surrounding air. At the edges of the isidium we find the same dark-walled cells with wavy outlines which may run some distance over the upper cortex in the form of hyphae. The cells of the under side have the same wavy outline and the same general appearance, and appear to show the same type of pore as in *P. praetextata*. The isidia are attached to the metathallus by a narrow stalk which may be centrally placed or more to one side. It contains few gonidia, and sometimes it is impossible even to make out these. The isidia occur singly at each break-through of the cortex, and they are small, flat, and not erect. But the upper and lower side are as different from one another as in *P. praetextata*. A very young isidium may be found growing literally in the shadow of the very oldest. They do not seem to increase the assimilating power of the lichen very much if at all.

# GENERAL CONCLUSIONS AND DISCUSSION.

The origin in the protothallus of the various tissues found later in the mature metathallus is described. The longitudinally running hyphae of the medulla run out into the meristematic plectenchyma from which arise the outer protecting tomentum, the cortex, the intergonidial hyphae, the mature medullary hyphae, and the hypothallus. There is no evidence that the gonidia arise inside the fungal hyphae. The structure of the rhizines is given in detail, special reference being made to the origin of the struts or bridges by which the longitudinal and other hyphae of the rhizine are united.

The following represents my view of the mechanism of the metabolic processes in *Peltigera*. Water with its inorganic solutes is brought into the lichen-thallus through the rhizines. It is then conducted to those portions of the thallus where it is required by means of the veins, or, if such are not differentiated, by means of the longitudinally running hyphae of the medulla. These hyphae are connected to the intergonidial network of hyphae which surround the gonidia by finer infragonidial hyphae. These intergonidial hyphae finally link on to the coarser subcortical hyphae from which the water passes directly to the cortical cells for transpiration. Sievers (30, p. 18) was able to show by experiment, in support of Zukal (38, pp. 37 and 38), that the cortex of the upper side of *P. canina* will not absorb water anything like as readily as the special organs of the under side; rather is it adapted to protect the thallus from desiccation—that is, to control transpiration.

I carried out some simple experiments with portions of *P. canina*, to find out which side was most actively transpiring. Upper and under side respectively were vaselined and in separate specimens. It was found that those vaselined below showed signs of desiccation in a very short time, clearly indicating the greater transpiratory activity of the upper cortex.

The cortical cells are either not directly or only very rarely directly in touch with the medullary hyphae, but for mechanical reasons they are firmly connected with the constituents of the hypothallus. Gaseous exchange takes place through the gaps or cyphellae on the under side of the thallus which lead by chimneys up to the gonidial layer.

The mechanical strength of the *Peltigera* thallus depends on the firm cementing together of the cell-rows of the cortex which is not interrupted normally. It also depends on the veins or bundles of hyphae which run longitudinally and which are closely connected together by anastomosing hyphae. These make the veins or longitudinal hyphae part of one system. The upper cortex is connected by straight hyphae with the thin layer of tough hyphae which makes up the hypothallus. The edges of the thallus are well secured against rupture (Pl. XXIX, Fig. 17).



The origin and healing over of cracks in the metathallus are described for *P. canina*, *P. polydactyla*, and *P. horizontalis*, as also in the case of *P. praetextata*. The origin of the isidia in cracks is due to some external or internal stimulus. The hyphae which are going to give rise to the isidium are differentiated very early from the medulla. These hyphae seem to be entirely absent in those species of *Peltigera* which do not possess isidia. They would therefore represent an important specific character of *P. praetextata*. For this reason I do not agree with Birger Nilson (20, p. 20) and Moreau (18, p. 115), who look upon isidiiferous individuals as biological forms only. In the isidium we get upper cortex, gonidial layer, loose medulla, and under cortex, the latter exhibiting pores which remind one of stomata in function, if not in structure. The cells of the primary upper and of the whole under cortex show wavy outlines.

The observations recorded in this paper show the very close co-operation which makes of the lichen not a dual organism consisting of algae and fungus, but one simple organism which consists of green tissue and non-green tissue. Morphologically we may look upon the lichen as a dual organism, but physiologically it is a simple organism. This point comes out most clearly when considering the isidium in its various stages of development.

Strato, Linkola, and Tobler consider that the algae or gonidia take the lead in the formation of the isidia whatever the systematic position of the individual may be on which they occur. It is obvious that isidia will not be formed if the conditions are definitely adverse. The conditions are, however, to be considered favourable only when they are favourable to the growth of both fungus and algae, i. e. to the lichen as a whole.

From the moment a crack is formed medullary hyphae grow up to surround the algae with special cells and to protect the latter against desiccation. The primary cortex thus formed always consists of the same type of cell and is always of the same thickness. Cortex and gonidia are physiologically parts of one and the same organism.

As I mentioned in a previous paper (8, p. 13), the fungus, by becoming a partner in the lichen business, has shaken off the traditional habits due to the morphological and structural differentiation of its saprophytic or parasitic ancestors, and has built up a new and mainly physiological tradition. The lichen fungus or the colourless part of the lichen is in such close and intimate touch with the surrounding conditions that the life of the whole lichen, not of the fungus alone, nor of the algae alone, depends on an immediate ontogenetic response to any change in these conditions. It acts in a definite way not because it is a fungus but because it is the colourless part of a green plant. The rhizine is not a fungal structure primarily, but the organ of a lichen. In *Peltigera* we have an example of the most complete kind of symbiosis. None of the terms usually suggested by various lichenologists is really applicable to the case of *Peltigera*, as they all imply

antagonism between gonidia and fungus. Schwendener speaks of parasitism (28, p. 88), Danilov of a fungus causing a disease on the gonidia (5, p. 33), Moreau of an alga causing a gall on the fungus (18, p. 125), Nienburg (19) and others of helotism, and Elenkin of endosaprophytism (9, p. 65). Reinke, however, considers that the lichen represents a consortium, implying a very intimate and mutually helpful co-operation between the constituents of the lichen. In the form of the lichen thallus he sees not a highly developed fungus, only containing algae as gonidia, but the result of the evolution of an assimilating, i. e. green, consortium (21, p. 75).

We know practically nothing of how a stimulus received by the leaf-blade of a green land-plant is passed on to its stalk, whereby the latter is led by growth and other means to place the leaf-blade in an attitude best suited for the particular function it usually carries out. I do not believe that the relation of leaf-blade to stalk in the green plant is any more intimate than that of algae to fungus in the lichen. The presence of pores on the under side of the isidia I take to be the expression of this intimate 'co-operation' which compels us to look upon the lichen *Peltigera* as physiologically a simple organism or consortium, and not a compound one. It is possible to generalize at this point and to say that the evolution of the lichen is strictly that of an autotrophic organism and not that of two organisms, one of which at any rate is heterotrophic. At the same time it is quite possible that this may not have been so in the very earliest stages of the evolution of the lichen.

Church (4, p. 267) considers that the structure exhibited by *Peltigera* is very much like that of a seaweed (though not of a type now extant in the sea), except that it has lost its assimilating cells, which have been replaced as regards function by intrusive green or other algae. To this I would raise objections by saying that the structure of *Peltigera*, with its extensive air-spaces and occasional pores for gaseous exchange, is that of a land-plant. I can only picture the development of these taking place in a race which began its evolution as such on the dry land. The lichens have descended from simple fungi long after the latter had become adapted to living, parasitically or saprophytically, on the land. I am not discussing here the question as to where the fungi came from originally. It is still possible for a green land-plant to enter fresh water or the sea and modify its land habit accordingly. It is difficult, if not impossible, for a red or brown seaweed of to-day to live completely on the land, as no modification of its sea-structure appears to be any longer possible along the proper lines. This has been said by Scott already (29, p. 201). Air-spaces and pores mean a long line of land ancestors, however simply organized the pores may be. These organs and the mechanical system in *Peltigera* are due to the sub-aerial habit above the substratum. So the development of the lichen as such has taken place entirely on the land from ancestors already used to the land.

*Peltigera canina* and *Ramalina fraxinea* are not the degenerate descendants of red algae, but as lichens exhibit forms which have been developed on the land from the association of the simplest fungus with green or blue-green algae.

#### SUMMARY.

1. Our knowledge of the structure of the mature metathallus of *Peltigera* and of the mechanism of the chief metabolic processes is reviewed, and also the development of the embryonic protothallus.

2. Growth, development, and function of the rhizines is described, special attention being paid to the origin at the tips of the hyphae of the transverse bridges or struts by which the longitudinal hyphae are connected.

3. True isidia are found apparently in *P. praetextata* and *P. lepidophora* only, soredia in *P. erumpens* and *P. scutata* only.

4. Cracks in the thallus, apparently due to external agencies, occur in several species of *Peltigera*, but are in all cases quickly healed over without leading to the formation of isidia, except in the case of *P. praetextata*.

5. In *P. praetextata* special infragonidial hyphae may break through the cortex in order to give rise to isidia, should no crack be formed in the cortex by external agencies.

6. The leaf-like isidia of *P. praetextata* exhibit a high physiological and anatomical differentiation along the lines of what is seen in the leaf of a typical dicotyledonous land-plant, even to the development of pores on the under side, resembling stomata.

7. The function of the isidia appears not to be reproductive, but in the first place probably photosynthetic.

8. Morphologically the lichen *Peltigera* may be considered a dual organism, but physiologically it is a simple autotrophic organism, of which the algal gonidia represent the green part and the fungus the colourless part.

9. The lichen *Peltigera* is a typical land-plant, and its ancestors must have developed as land-plants from the very earliest stages, probably even from the very first lichen stage as such.

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## EXPLANATION OF PLATES XXVIII-XXXI.

Illustrating Professor Darbishire's paper on the Structure of *Peltigera* with Especial Reference to *P. praetextata*.

### PLATE XXVIII.

Fig. 1. *Peltigera praetextata*. Vertical section of metathallus, showing upper cortex, gonidial layer, infragonidial hyphae, longitudinal hyphae, and hypothallus. A rhizine arises from the longitudinal hyphae and the course of the hyphae in the rhizine near its point of attachment to the metathallus can be seen.  $\times 50$ .

Fig. 2. *Peltigera praetextata*. A few hyphae taken from Fig. 1 and showing the angle which the hyphae of the rhizine make with those of the medulla.  $\times 300$ .

Fig. 3. *Peltigera canina*, f. *spongiosa*. Vertical section of protothallus and metathallus. The distribution of tissues is the same as that shown in Fig. 1, the gonidial layer being marked in black. The latter is interrupted towards the right by a crack which has healed over. The two black lines running from cortex to hypothallus show the appearance of the tissues between the veins, where loose tissue is found from hypothallus to gonidial layer. Rhizines are seen on the under side in various stages of development. The second from the left has overgrown an old thallus of *Peltigera*, and between the second and third from the left a small bit of moss leaf is seen.  $\times 15$ .

Fig. 4. *Peltigera horizontalis*. A typical compound rhizine consisting of numerous strands united together, and each spreading out on reaching the solid substratum. The neck is slightly contracted.  $\times 15$ .

Fig. 5. *Peltigera horizontalis*. Short rhizine which is spreading out over a moss leaf.  $\times 15$ .

Fig. 6. *Peltigera canina*. Rhizine, the tip of which has penetrated into loose soil. Single hyphae arise from its sides.  $\times 75$ .

Fig. 7. *Peltigera canina*. Rhizine, the tip of which has spread out over a moss leaf.  $\times 15$ .

Fig. 8. *Peltigera horizontalis*. A compound rhizine spreading out in damp atmosphere before it has reached the substratum.  $\times 15$ .

Fig. 9. *Peltigera canina*. Conical embryonic rhizine near margin.  $\times 25$ .

Fig. 10. *Peltigera canina*. Details of hyphae at apex of the young rhizine shown in Fig. 9. The fusions at the apices of the hyphae and the commencing differentiation of the inner longitudinal hyphae from the outer loose hyphae can be seen.  $\times 650$ .

Fig. 11. *Peltigera horizontalis*. End of the hyphal network of a rhizine overgrowing a moss leaf as shown in Fig. 5. Apical fusions and the origin of branches are shown. The dotted outline indicates that the hypha thus shown has penetrated the outer walls of the moss leaf.  $\times 650$ .

Fig. 12. *Peltigera canina*. The apex of a fine strand from the rhizine growing over moss shown in Fig. 7.  $\times 650$ .

Fig. 13. *Peltigera canina*. Transverse section of mature rhizine. The inner and larger hyphae are seen in the centre running longitudinally and provided with interhyphal air-spaces. Then follow

the smaller longitudinal hyphae. On the outside are the loose hyphae which are continuous with the hypothallus of the main metathallus.  $\times 300$ .

Fig. 14. *Peltigera canina*. A few inner hyphae of a mature rhizine shown in transverse section. Four fusions between hyphae are shown, also the vessel-like interhyphal passages.  $\times 1,000$ .

Fig. 15. *Peltigera canina*. A few inner hyphae of a mature rhizine shown in longitudinal section. The right-angle fusions which act as struts are shown.  $\times 1,000$ .

PLATE XXIX.

Fig. 16. *Peltigera horizontalis*. A small colony of *Nostoc* cells, the surrounding mucilage of which has been invaded by branches from the intergonidial hyphae.  $\times 1,000$ .

Fig. 17. *Peltigera horizontalis*. The mature edge of the metathallus, which has ceased to grow, and shows the close cementing together of the hyphae to secure protection against rupture.  $\times 300$ .

Fig. 18. *Peltigera polydactyla*. A healed crack, showing the abrupt endings of the old cortex where it has been broken through. The healing of the wound is due to the medullary hyphae.  $\times 300$ .

Fig. 19. *Peltigera praetextata*. Vertical hand section of the protothallus showing the general course of the hyphae. The medullary hyphae are seen to radiate out beyond the gonidial layer (here shown quite black) and to give rise to the extensive tomentum above and below.  $\times 75$ .

Fig. 20. *Peltigera praetextata*. The protothallus as shown in Fig. 19, but more highly magnified. The course of the hyphae is in reality slightly more irregular than is shown in this diagram.  $\times 500$ .

Fig. 21. *Peltigera polydactyla*. An old healed crack under which gonidia have penetrated again.  $\times 300$ .

Fig. 22. *Peltigera polydactyla*. Cracks in the old cortex indicating where a break-through is probably going to take place.  $\times 300$ .

Fig. 23. *Peltigera polydactyla*. General view of healed crack.  $\times 75$ .

Fig. 24. *Peltigera polydactyla*. The same crack in detail. The arrows indicate the limits of the primary cortex.  $\times 300$ .

Fig. 25. *Peltigera polydactyla*. General view of a surface wound, showing the interruption of the cortex and the gonidial layer (shown in black).  $\times 75$ .

Fig. 26. *Peltigera polydactyla*. A portion of the wound shown in Fig. 25. The medullary hyphae have formed a new cortex to cover the exposed gonidial layer.  $\times 300$ .

Fig. 27. *Peltigera canina*, f. *spongiosa*. The healing of a crack by medullary hyphae growing into the gap and beyond on to the cortex on the opposite side of the wound.  $\times 300$ .

PLATE XXX.

Fig. 28. *Peltigera praetextata*. A break-through in the cortex being healed over by hyphae from the medulla. All the gonidia are already well covered over.  $\times 300$ .

Fig. 29. *Peltigera praetextata*. Vertical section of metathallus. A few hyphae of medullary origin are seen breaking through the cortex in preparation to forming isidia. The apical cell of the largest hypha shows the branching which results in the formation of the cells with wavy outlines.  $\times 300$ .

Fig. 30. *Peltigera praetextata*. An obliquely transverse section of a group of medullary hyphae breaking through the cortex in order to give rise to isidia. This section practically represents the hyphae of Fig. 29 in transverse view.  $\times 300$ .

Fig. 31. *Peltigera praetextata*. Group of algae which have been surrounded by medullary hyphae which, though still well inside the thallus, are already beginning to show the wavy outlines characteristic of the mature protective sheath of the isidia.  $\times 500$ .

Fig. 32. *Peltigera praetextata*. Two young isidia still covered by the original layer of small dark-walled cells, mostly exhibiting wavy outlines. The connexion between the big cells of the stalk and the cells of the medulla is shown. The abrupt ending of the cortex at the point of its break-through can be seen clearly, and also the continuous new layer which stretches across the gap from cortex to cortex. Both the isidia, though still almost spherical, are already showing, by the position of the gonidia, that the upper side of the mature isidium is going to be to the right of the reader.  $\times 300$ .

Fig. 33. *Peltigera praetextata*. A young isidium well below the level of the cortex. Medullary hyphae have given rise to the cells enclosing the gonidia, and these already show the wavy outlines in the early stages of their development.  $\times 300$ .

Fig. 34. *Peltigera praetextata*. An open gap which has been completely healed over. On the slope in the gap gonidia and hyphae can be seen to be combining to grow out to form an isidium. This is shown in its very earliest stage of development.  $\times 300$ .

Fig. 35. *Peltigera praetextata*. An early stage in a break-through of the cortex through the gap of which hyphae are seen to be growing. One or two gonidia can be seen to have suffered by the exposure due to the cracking of the cortex. Two small colonies of *Nostoc* are seen on the level of the upper cortex.  $\times 300$ .

Fig. 36. *Peltigera praetextata*. A group of isidia, all of which show the same orientation of their tissues. Again the discontinuity of the old cortex can be observed and the continuity of the new layers. There is no gaping wound. The details of the structure of the isidium can be seen from the text. But the gradual emergence of the mature cortex of the upper side from the primary dark-walled cortex of the embryonic condition should be noticed. On the right a quite small and young isidium has just started growth.  $\times 150$ .

#### PLATE XXXI.

Fig. 37. *Peltigera praetextata*. A full-grown isidium in surface view after being torn from its point of attachment to the metathallus.  $\times 20$ .

Fig. 38. *Peltigera praetextata*. Marginal portion of a mature isidium. The cortex of the under side, interrupted on the right by a pore, is continued to the left over the more delicate inner tissues which gradually emerge as the cortex proper of the upper side of the mature isidium. The marginal cells run out over the upper cortex as dark-walled hyphae.  $\times 300$ .

Fig. 39. *Peltigera praetextata*. Surface view of the margin of the upper side of an isidium, showing the secondary cortex of cylindrical cells emerging from between the dark hyphal outrunners of the primary cortex.  $\times 300$ .

Fig. 40. *Peltigera praetextata*. Surface view of margin of the upper side of a mature isidium showing the secondary cortex emerging gradually from between the dark cells of the primary cortex.  $\times 300$ .

Fig. 41. *Peltigera praetextata*. Surface view of the secondary cortex of a mature isidium.  $\times 300$ .

Fig. 42. *Peltigera praetextata*. Surface view of the margin of the under side of a mature isidium, showing the cells with wavy outlines.  $\times 300$ .

Fig. 43. *Peltigera praetextata*. Surface view of a more central portion of the under side of an isidium, showing the distribution of pores, of which five can be seen.  $\times 300$ .

Fig. 44. *Peltigera praetextata*. Surface view of under side at stalk end of isidium.  $\times 300$ .

Fig. 45. *Peltigera praetextata*. A single pore in surface view from Fig. 43.  $\times 600$ .

Fig. 46. *Peltigera praetextata*. A single pore in surface view from an isidium as shown in Fig. 49.  $\times 600$ .

Fig. 47. *Peltigera praetextata*. An oblique section through the under side of the growing portion of an isidium. On the left are the loose hyphae of the medulla, which on the right are combining to form the continuous cortex of the under side.  $\times 600$ .

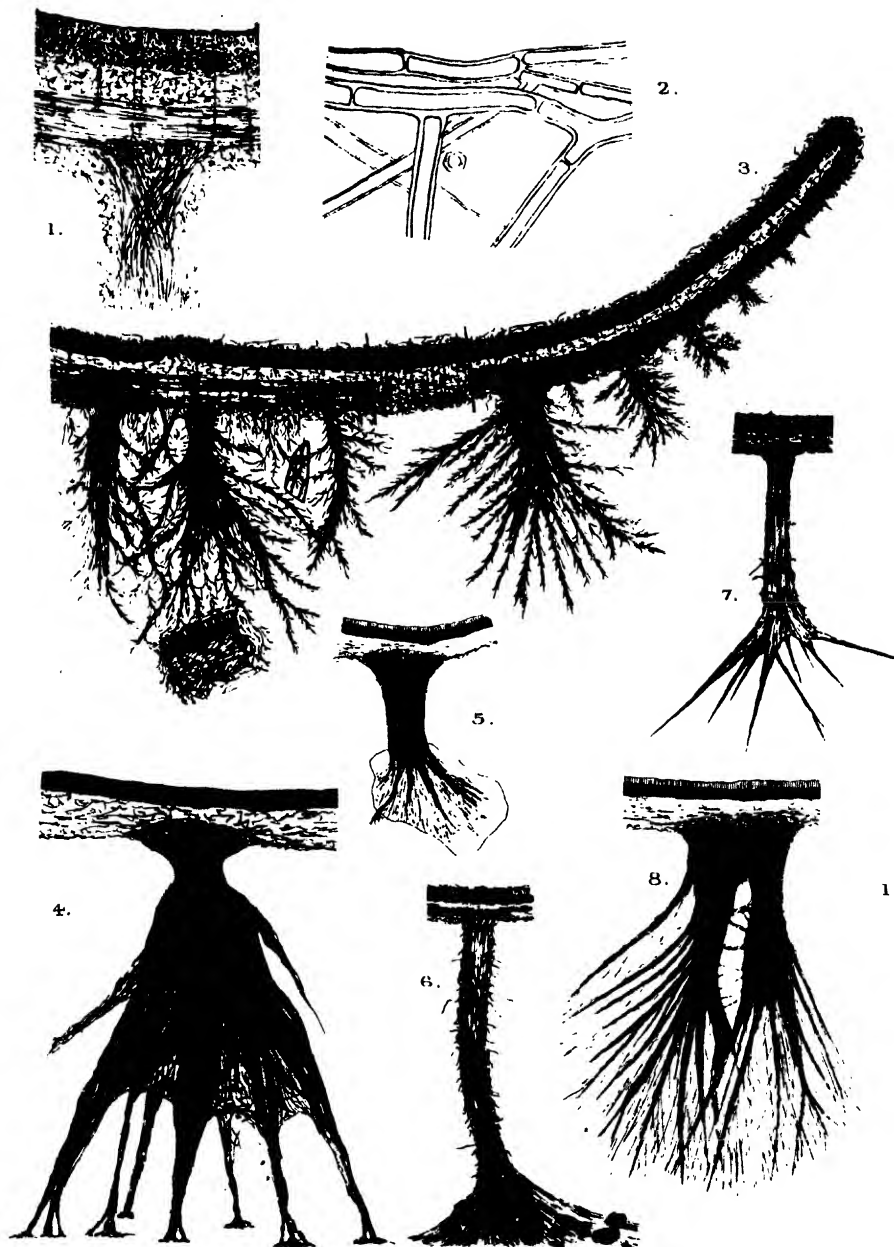
Fig. 48. *Peltigera praetextata*. Crowded hyphae preparing to break through the upper cortex of the metathallus from within before any crack has been formed. Isidia will be formed when the break-through is complete.  $\times 600$ . Microphotograph.

Fig. 49. *Peltigera praetextata*. Microphotograph of vertical section of mature isidium, showing upper cortex, gonidial layer, loose medulla, and lower cortex with a pore in transverse section of the opening. Three cells to the right, one can be made out cut longitudinally.  $\times 300$ .

Fig. 50. *Peltigera praetextata*. A microphotograph of the under side of an isidium such as is shown in Fig. 43.  $\times 600$ .

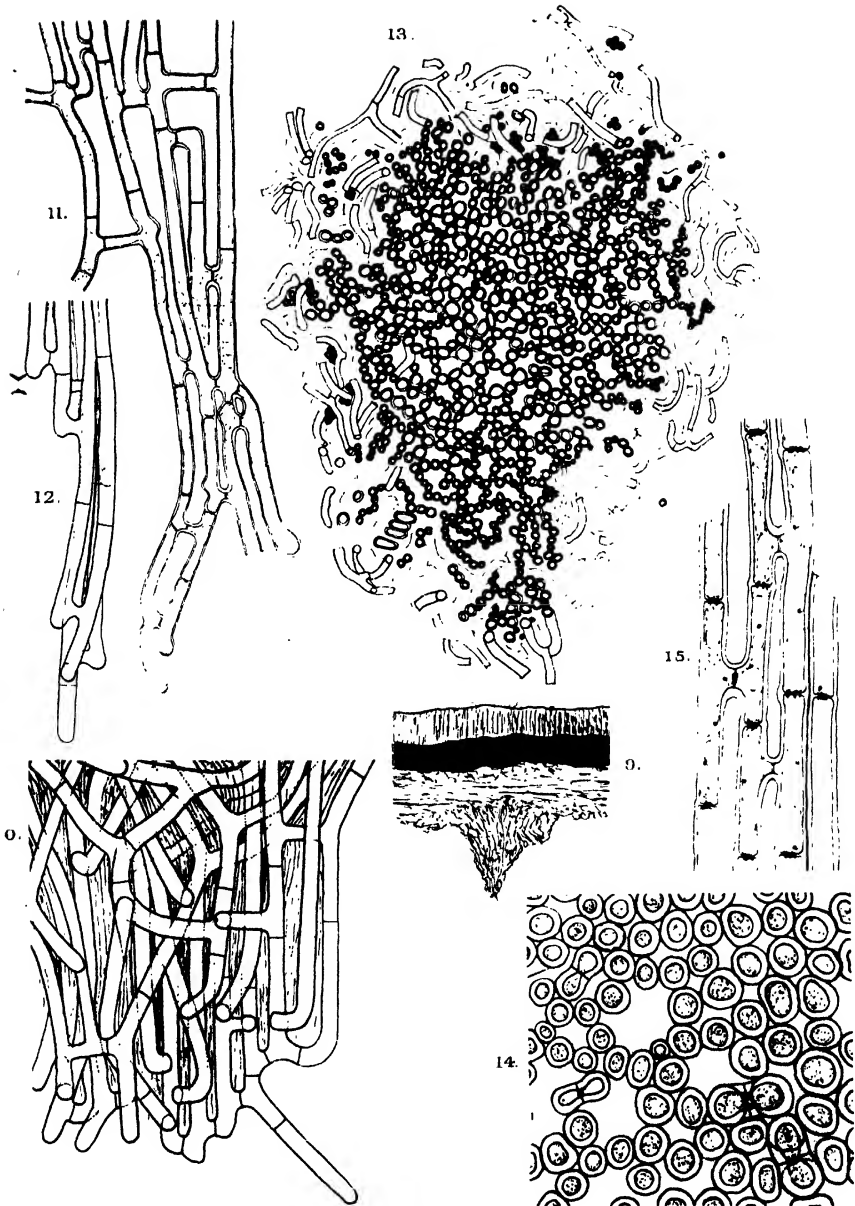


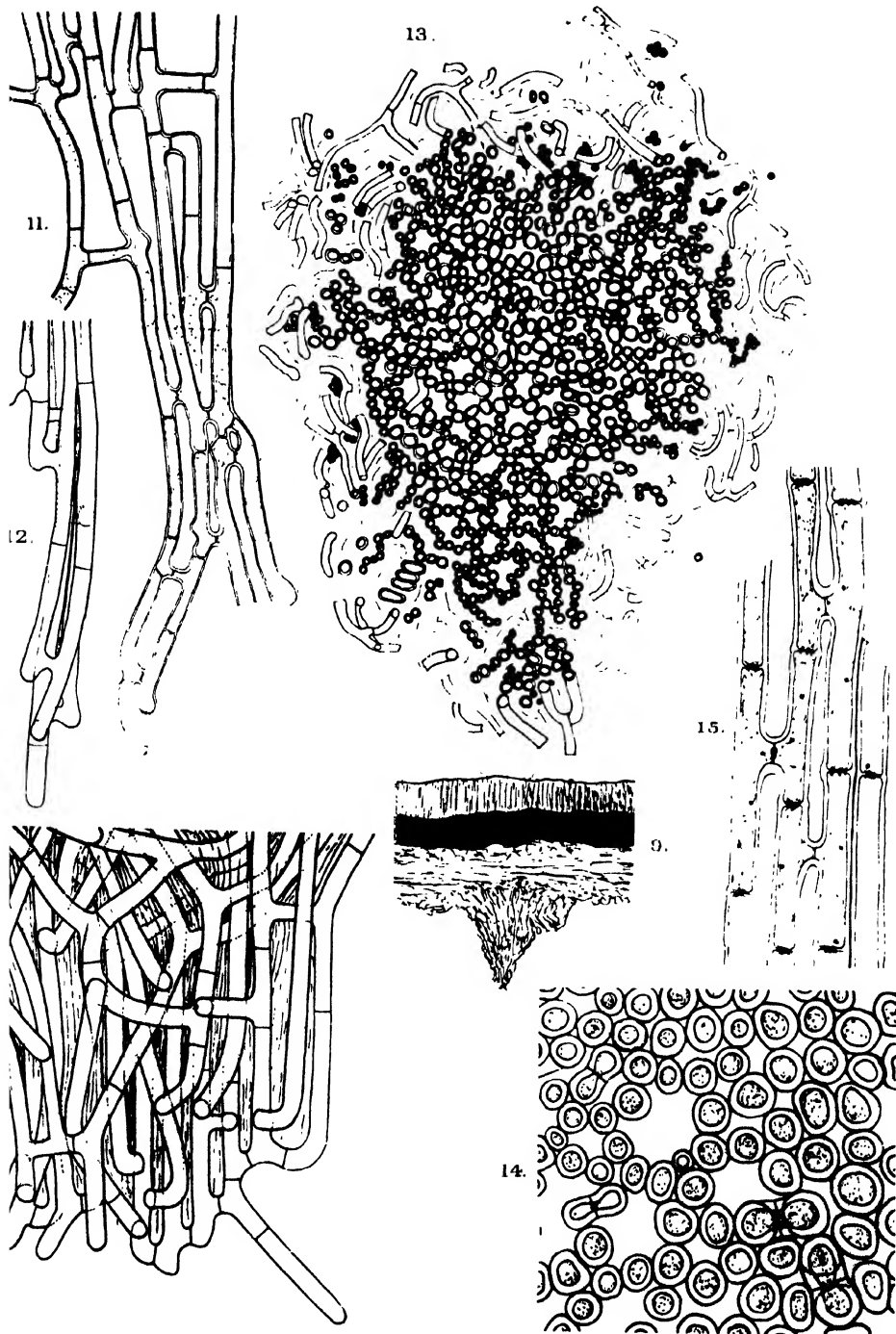


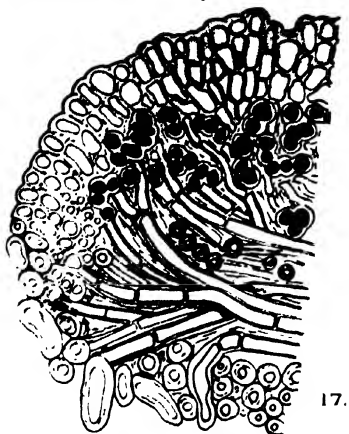


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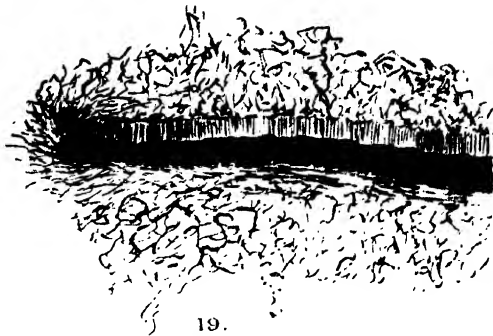
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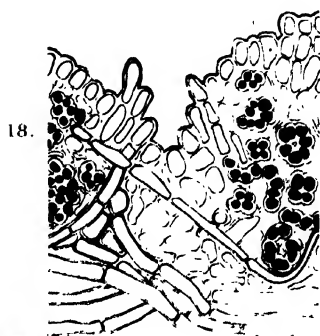
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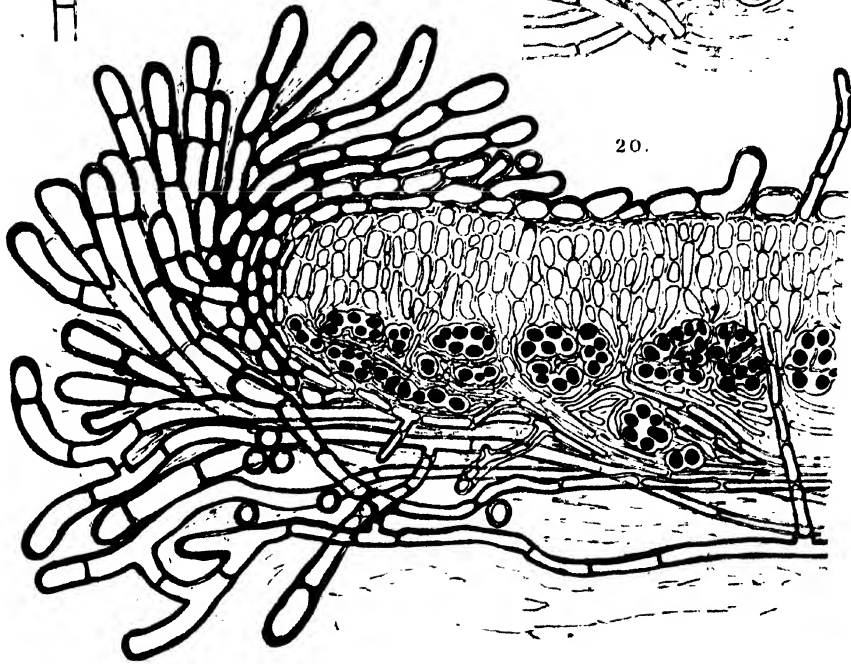
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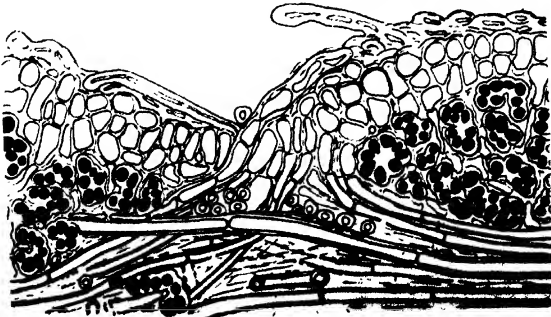
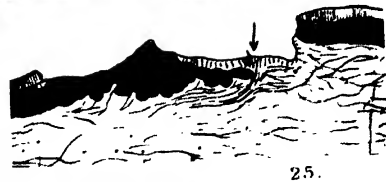
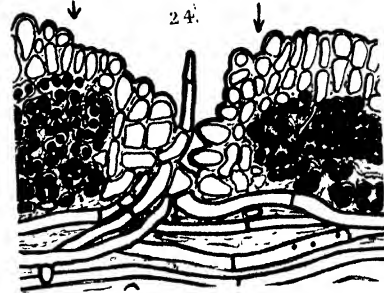
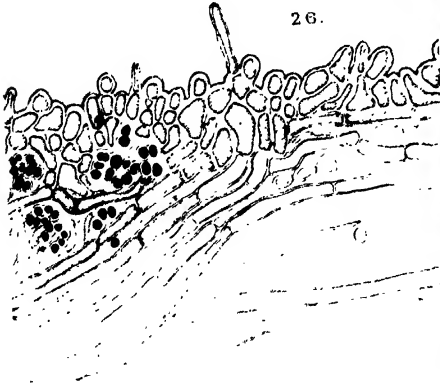
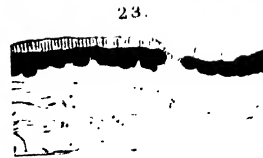
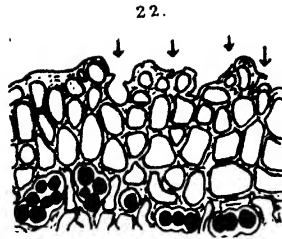
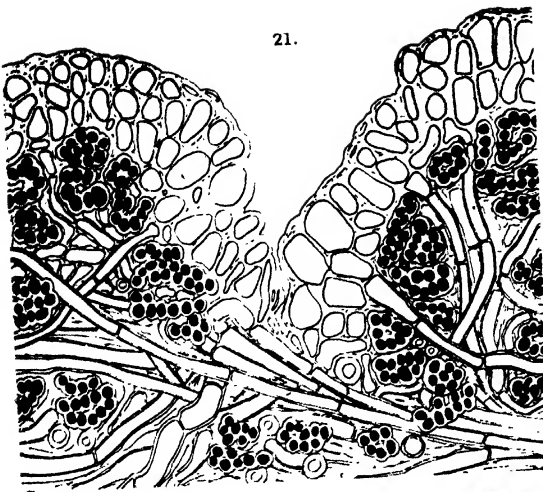
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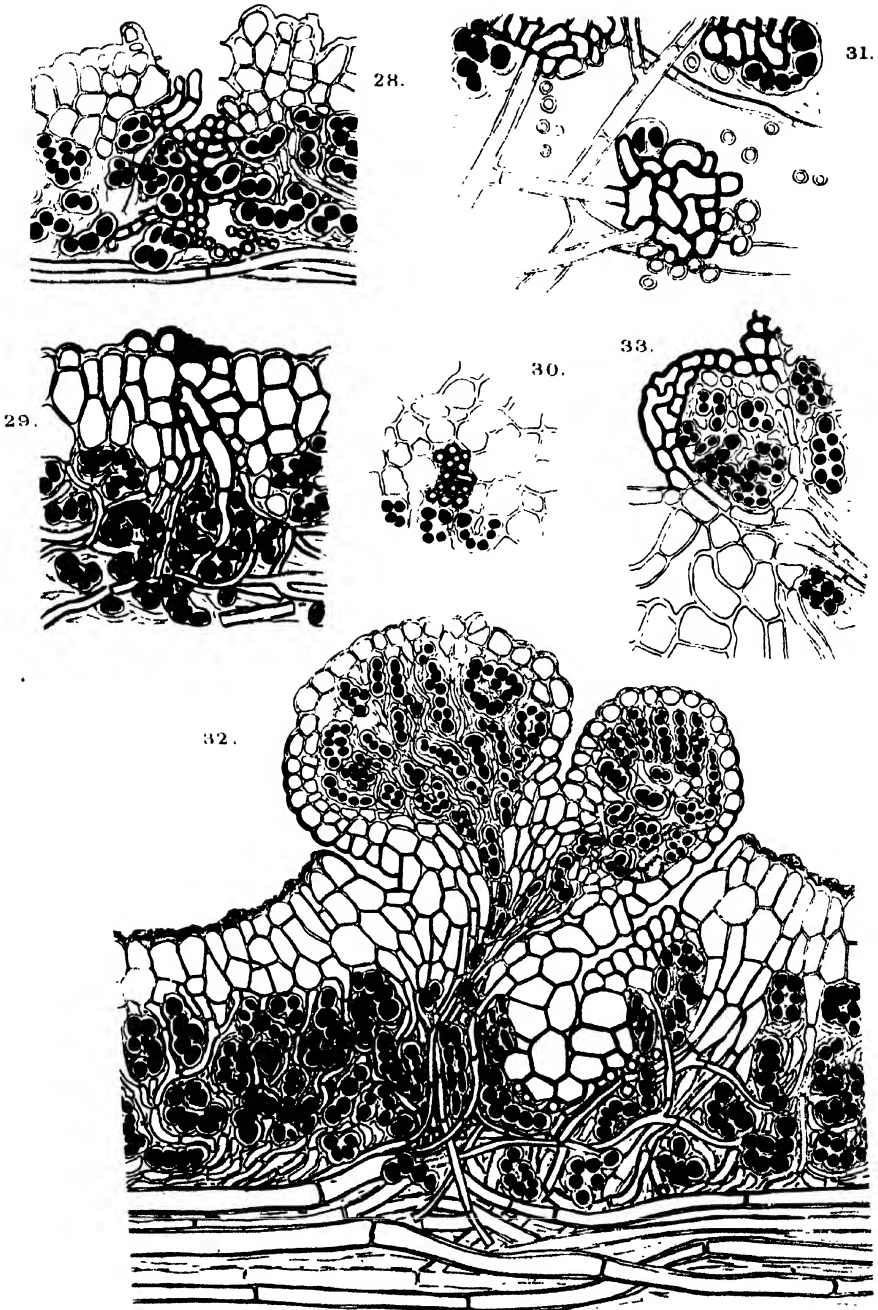


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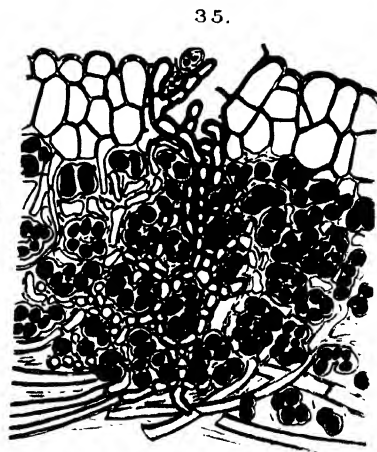
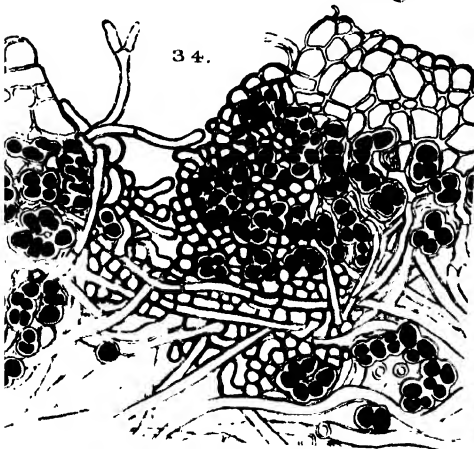
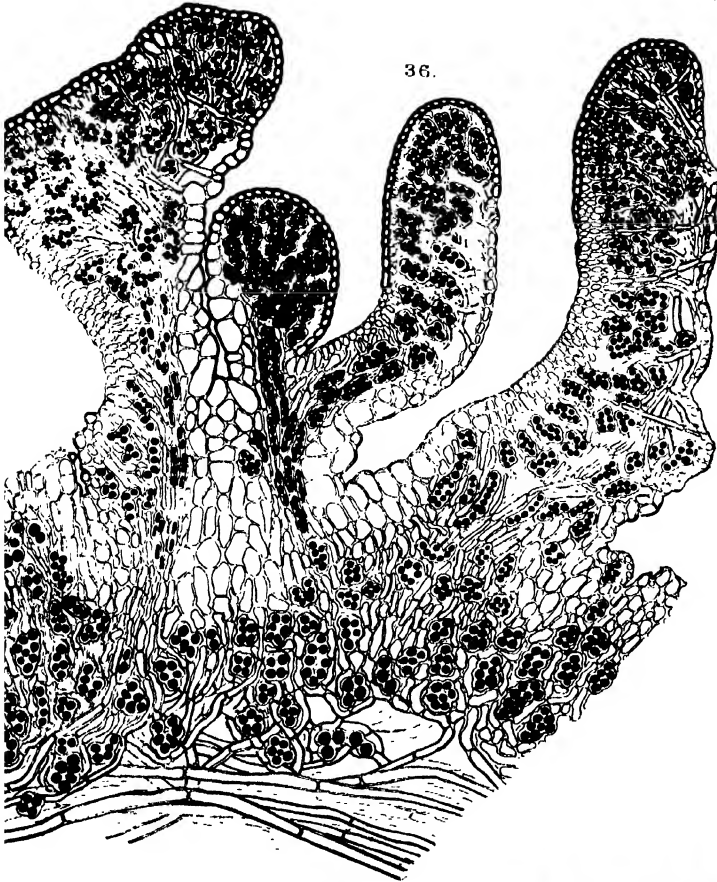
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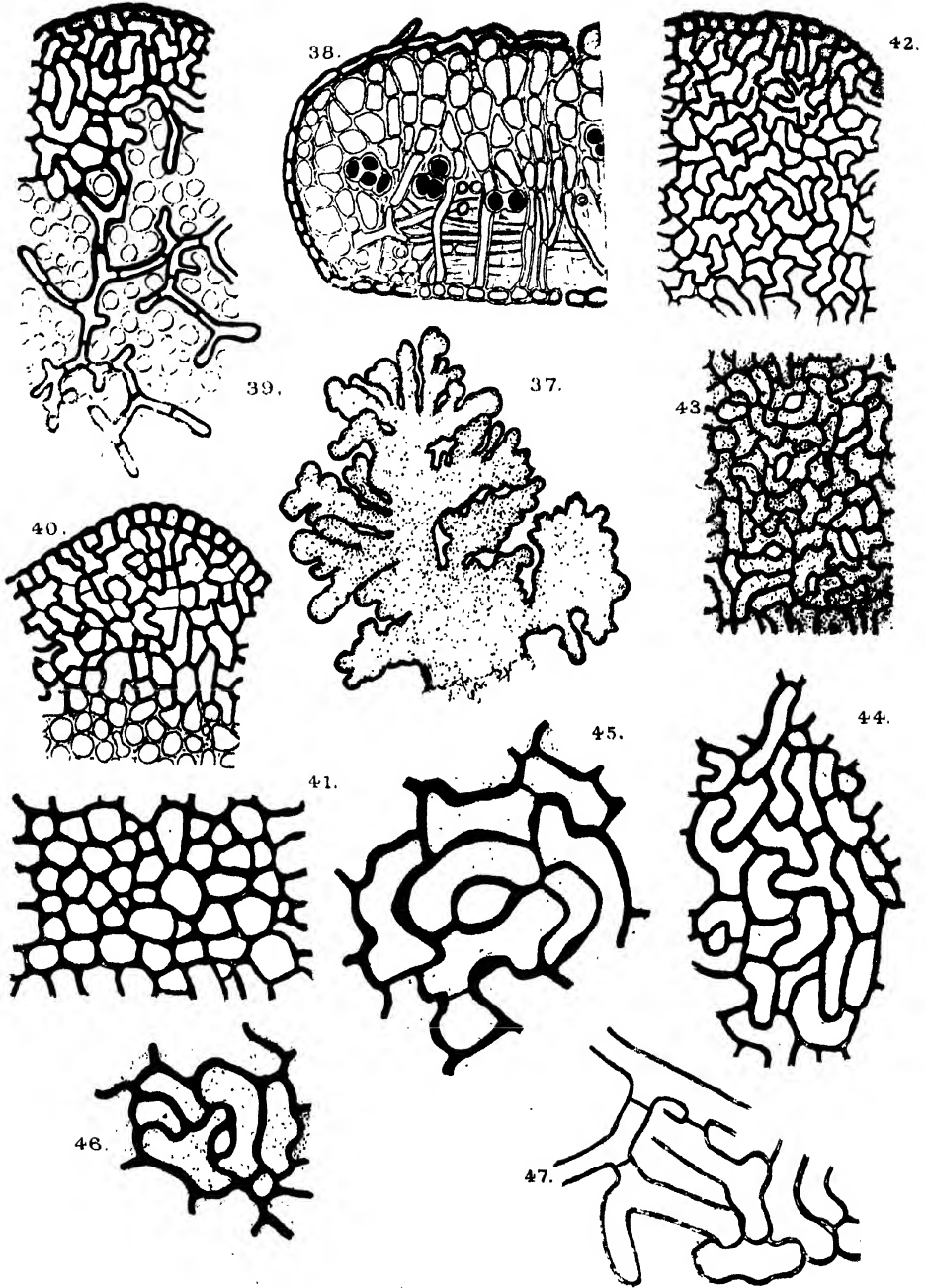
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48.



# On Two Obscure Diseases of Cotton.

BY

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AND

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With Plate XXXII and six Figures in the Text.

IN 1912 Farquharson (5), Mycologist in Nigeria, observed a disease of Native cottons (*Gossypium peruvianum* and *G. vitifolium*) which he described under the name of 'Leaf Curl', for the leaf margin was curled upwards. The veins of affected leaves showed a peculiar puckering, while the leaves themselves were stunted or distorted, and often chlorotic, though chlorosis was not universal. Flowering was restricted, but death did not result. Okra (*Hibiscus esculentus*) was similarly affected. In the following year he described (6) the symptoms more fully. The puckering or intumescences on the leaf veins were always on the under surface and were due to 'local access of spongy parenchyma', the palisade cells appeared to be modified, but the epidermis remained intact. He noted that cotton grown in the humid forest regions near to the coast was unaffected.

Early in 1924, while making a survey of cotton farms in the Oyo and Abeokuta provinces of Southern Nigeria, it became evident to one of us that 'Leaf Curl' was of widespread occurrence. The studies reported in the present paper were accordingly initiated.

In September of the same year a novel feature was observed on some American cotton (*G. hirsutum*) at Oyo. The leaves showed a downward rolling of their margins, the under side was glazed and slightly brownish in colour, while the upper surface appeared to be a duller green than normal. In severe cases the leaf surface cracked and holes developed.

In view of the great variety of symptoms to which a single disease may give rise on different varieties, or even on different individuals of the

same variety, it was at first considered possible that the two diseases, the upward curl of the leaf margin on Native plants and the downward roll on American, might prove to be identical. Investigation has shown that this is not the case. It is proposed therefore to follow Farquharson, retaining the name 'Leaf Curl' for the disease he originally described upon Native plants, and to employ the term 'Leaf Roll' for the disease recently discovered on American plants.

It has been found, however, that either disease may appear on either host, but while 'Leaf Curl' is very prevalent on Native cotton, it is in our experience rare upon American; on the other hand 'Leaf Roll' is much more frequent on American cotton than on Native.

### LEAF CURL.

*General Description.* Affected Native plants (*G. peruvianum* and *G. vitifolium*) show a strong upward curl of the leaf margin (Pl. XXXII, cf. Figs. 1 and 2). This enables severely diseased plants to be easily detected in the field, as in the case of 'Leaf Curl' of the potato. The resemblance to potato 'Leaf Curl' is further emphasized by the fact that the leaves feel thicker and more brittle than normal, and emit a rattling sound when the plant is shaken.

Closer examination reveals the presence of enations—the most characteristic feature of the disease—on the lower surface.

These may arise at any stage in the development of the leaf or of the plant. In slightly affected leaves they occur as minute outgrowths at a few points on the smaller veins in the form of papillae, but in severe cases as a regular thickening all along the main and secondary veins.

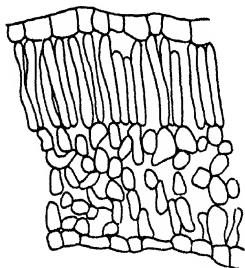
Not infrequently these net-vein enations give rise to minute foliar structures (Pl. XXXII, Fig. 2 *a*) which may attain a breadth of half a centimetre. The upper surface of the leaf is usually normal, though in certain cases it may be slightly mamillated.

The colour of affected leaves is usually darker than normal, but the leaf may be mottled to a chlorotic and dark green mosaic, or (in cases) even 'savoyed' light and dark green. In severe cases the bracts may also be affected by net-vein enations, but no other abnormalities have been observed in other parts of the plant.

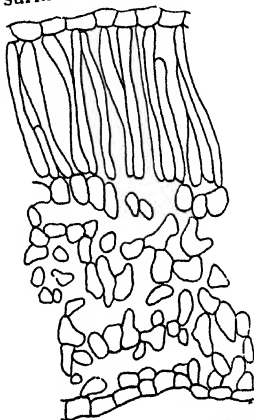
In American cotton (*G. hirsutum*) the symptoms of 'Leaf Curl' are somewhat different. The internodes are shortened, giving the plant a 'bunchy top' appearance. Affected leaves are greatly stunted and distorted by 'blisters', while the colour is 'savoyed' a light and dark green. In rare cases enations occur as minute foliar growths on the *upper* surface of the leaf, in contrast to the enations of affected Native plants, which always occur on the *lower* surface. These abnormalities produce

a striking change in the appearance of the plant, so that diseased plants are readily noticed in the field. American plants affected by 'Leaf Curl' are strongly reminiscent of Cook's (3) figures of 'stenosis' in cotton.

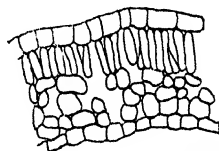
*Histology.* Microscopically, the most striking feature of affected leaves is their thickness (Text-figs. 1 and 2); diseased leaves are approximately half as thick again as normal leaves. A section shows that the lamina is covered with indentations. This causes the veins to appear embossed in macroscopic view, and explains the slightly mamillated appearance of the upper surface. The palisade cells are longer than



TEXT-FIG. 1. Transverse section of normal cotton leaf.



TEXT-FIG. 2. Transverse section of cotton leaf affected by Leaf Curl.



TEXT-FIG. 3. Transverse section of cotton leaf through a thin area in the mosaic type of Leaf Curl.

normal, while the spongy mesophyll is more bulky and irregular. There appears to be a difference in the staining reaction of healthy and curled leaves; healthy leaves are strongly stained by erythrosin (70 per cent. alcoholic), but the staining of curled leaves, using precisely the same method, is much fainter.

A section of a mottled mosaic type of cotton leaf (Text-fig. 3) shows remarkable variations in thickness, some areas being thick and others thin. Woods (12) was apparently the first to demonstrate the histological modifications that occurred in virus-affected leaves. He pointed out that the yellow areas were thin, and the dark green areas thicker. The former he considered diseased and the latter healthy. Chapman (2), while agreeing that the thick green areas might be healthy, showed that their structure was somewhat modified, presumably as a result of the increased functioning thrown upon them. Dickson (4), on the other hand, suggested that these dark green areas were the result of slight, or late, infection, which has stimulated them to hypertrophy.

Now the mosaic type of cotton Leaf Curl agrees in having thin yellow

and thick green areas. But the more usual type consists entirely of dark green areas, and hypertrophy has proceeded so far, in certain cases, that foliar structures have grown out. The thin yellow areas are in this case wholly absent. Whether their suppression is due to high temperature, which Dickson (4) has demonstrated tends to diminish the symptoms in tobacco mosaic, or to some other factor must await further investigation.

In this connexion it may be significant that tobacco plants in Nigeria frequently show precisely the same kinds of enations and foliar structures as have just been described from cotton plants. It is of interest to note that a tobacco disease which was characterized by the presence of enations and 'ear-shaped outgrowths' was first described by Raciborski (11) from the Dutch East Indies, and named by him 'Kroepoek'. More recently Jensen (7) has again described 'Kroepoek', which he distinguished from the mosaic disease of tobacco for two reasons: firstly, because there is no recovery from 'Kroepoek' in cases where mosaic plants have recovered; and, secondly, because there is no evidence of the grouping of affected plants in the field, as there is in the case of the mosaic disease.

In view of this, and of the similar evidence of complexity in the virus diseases of potato which Quanjer (10) has lately summarized, it is well to regard with suspicion the identity of the outgrowth type of cotton 'Leaf Curl' and the mosaic type of 'Leaf Curl'. We have as yet no critical evidence on this point.

There does not appear to be any other striking abnormality in curled plants; in particular there seems to be nothing approximating to phloem necrosis or starch accumulation.

A microscopic examination of an affected American leaf shows variations in thickness, &c., essentially similar to those found in the Native mosaic type of 'Leaf Curl'.

*Infectivity.* Evidence of infectivity was sought in several ways. Firstly, diseased plants have been kept under observation for upwards of eighteen months. In no case has recovery been observed.

Secondly, the stems of severely affected plants were cut 2-3 nodes above the cotyledonary node. The new growth that developed subsequently was all affected.

This evidence, while not in itself positive proof of the infectious nature of the disease, yet renders its causation by climatic factors improbable, in view of the markedly inconstant nature of the physical environment in this part of the world.

Thirdly, dormant buds from diseased plants were budded on to healthy stocks. Plants affected with 'Leaf Curl' were potted at Ibadan, their leaves were stripped off, and they were transported to a field of cotton in the Ilugun forest, a distance of twenty miles, where they were budded.

\* The stocks were cut back about a foot from ground level, a few leaves

being left intact and the bud introduced about six inches below the cut. The field was selected for its almost complete absence of 'Leaf Curl'. It was ascertained that there were no affected plants within forty yards of the plants used as stocks, and throughout the season there have probably been less than ten cases of 'Leaf Curl' in the whole field, all of which were rogued out as soon as they were observed.

Five out of twenty plants produced shoots from the buds taken from diseased plants. All the leaves on these shoots were severely diseased. About a month later the leaves on the shoots originating from the stocks were similarly affected. No plants developed the disease in the vicinity of the budded plants, but as there was the possibility of insect or other accidental infection, buds from diseased plants were budded on to healthy plants growing in cages; as the results confirmed those obtained in the open, it may, we think, be concluded that the disease is infectious.

Attempts were also made to induce the disease by means of inoculation. Several series of experiments were set up, using extracted juice and macerated leaves of curled plants, but in no case has infection by inoculation been obtained.

To sum up, the infectious nature of the disease is indicated by budding, and as no organism has been found associated with the disease, it seems that the 'Leaf Curl' of cotton must be classed with virus diseases. The abnormal internal anatomy, moreover, has a striking resemblance to those abnormalities encountered in other virus diseases.

*Effect of Environment.* Farquharson noted that cotton was not affected by 'Leaf Curl' in the moist belt near the coast, whereas at Ibadan, some seventy miles up-country, the disease was very prevalent. As very little cotton is grown near the coast it is difficult to gauge how much weight is to be attached to his observation. We have not noticed any very marked place effect, but the time of year seems to affect pronouncedly the rate at which the disease spreads.

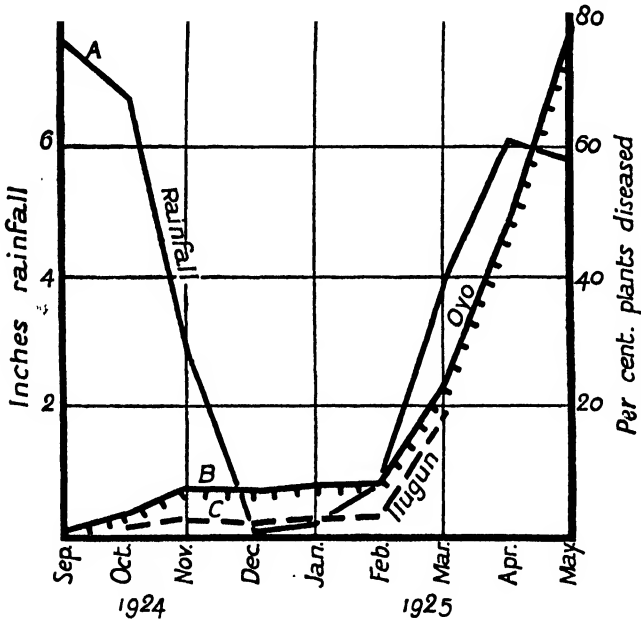
The rains in the part of Nigeria (Oyo and Abeokuta provinces) in which we have been at work generally continue from about April to October, frequently with a break about August. The months of January, February, and March are especially dry. The cotton is commonly planted about July, and thus makes its growth under conditions of very high humidity, while fruiting takes place in the dry weather.

When cotton is planted much before the normal time of planting in July, nearly the whole population may become affected with 'Leaf Curl'. Thus we have found (Table I) that when cotton is planted in May, nearly every plant became diseased within four months from the date of planting. It will be seen that the percentage of plants affected diminished as the normal time of planting is approached. It was observed that Jassids were very numerous about May and June.

TABLE I. *Showing Percentage of Population affected by 'Leaf Curl' within Four Months of Time of Planting.*

Month of Planting (1924).	Percentage of Population diseased.	Number of Plants.
May	92.0	272
June	8.5	3,141
July	0.6	3,774

Much of the cotton grown is left standing and not uprooted in the spring. We have kept two fields of cotton under observation (1924-5),



TEXT-FIG. 4. Showing mean rainfall (1914-23) at Ibadan (curve A) and percentage of plants affected by 'Leaf Curl' at Oyo (curve B) and Ilugun (curve C).

one in the Ilugun forest, and the other in bush country near the town of Oyo. The percentage of plants (Text-fig. 4) affected by 'Leaf Curl' was recorded about the middle of each month. The mean rainfall at Ibadan for the ten-year period 1914-23 is also shown in order to indicate the general change of climate in this part of the country. It will be seen that the disease spread very slowly until the advent of the spring rains. It may perhaps be concluded from this, and from the large proportion of plants that were affected by 'Leaf Curl' in the May-sown cotton (Table I), that the disease is normally a serious menace only in the spring months.

Now it was again (1925) noticed that Jassids were especially abundant in May, and as it appeared probable that these insects might act as vectors,



some plants were grown in muslin cages and Jassids collected from diseased plants introduced. The insects were placed in three cages, and three cages were left as controls. 'Leaf Curl', however, appeared on none of the plants. It would appear that if these insects do act as vectors, there must be some factor at work in the open that did not come into operation in the cages. It may be that none of the insects used happened to be infected, but it is also possible that the shade inside the cages suppressed the symptoms of the disease. The latter seems improbable, for, as already reported, plants could be infected by 'budding' in cages, though possibly the lesions were somewhat less marked than in the open. It will be evident, however, that our results are open to the interpretation that infection takes place at an approximately uniform rate throughout the year, but that there is a tendency for the expression of the disease to be suppressed until the spring.

*Possible Alternate Hosts.* We have attempted to bud a number of the indigenous Malvaceae on to cotton, but without success. It is interesting to note that members of the Malvaceae other than *Gossypium* appear to favour the development of certain symptoms in preference to others. Thus *Hibiscus esculentus* and *Sida* spp. usually show the net-vein symptoms in preference to mottling, while many wild species of *Hibiscus* are markedly mottled. *Urena lobata*, on the other hand, seems to be either mottled or to have net-vein enations impartially. It has been observed that *Triplochiton Johnsonii*, though a member of the Sterculiaceae, frequently exhibits the net-vein symptoms.

*Geographical Distribution of 'Leaf Curl'.* 'Leaf Curl' was first described by Farquharson from the Ishan district (SE. Nigeria), and it has since been found common in the Oyo and Abeokuta provinces (SW. Nigeria), while specimens have reached us from Zaria (N. Nigeria). It therefore seems probable that 'Leaf Curl' occurs throughout Nigeria.

From an examination of dried specimens sent to the Imperial Bureau of Mycology from the Anglo-Egyptian Sudan—a method of diagnosis admittedly open to criticism—we are of the opinion that 'Leaf Curl' occurs there also.

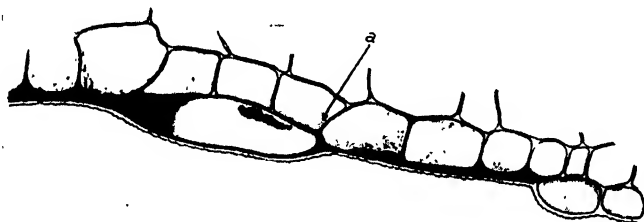
#### LEAF ROLL.

*General Description.* American plants affected by 'Leaf Roll' at first show a crimping of the leaf margin, and later a pronounced downward roll of the edge of the lamina. The upper surface of the leaf is of a duller green than normal, while the under surface presents a glazed and somewhat brownish corky appearance in contrast to the green pubescence of healthy leaves (Pl. XXXII, Figs. 3 and 4). The veins are sharply delimited on this glazed surface.

If leaves at this stage are carefully subjected to a slight tension along the lamina, the lower surface cracks while the tissues beneath remain whole, just as 'perished' rubber behaves under tension, and due, as will be seen later, to the same causes.

In the case of severely affected leaves the glazed surface cracks between the leaf-veins, carrying the tissues beneath with it, and thus originate peculiar irregular holes, reminiscent of the fenestrations which occur in certain plants, notably *Monstera deliciosa* and some water plants (1). These fenestrations may occur to such an extent that the leaf is subdivided into a coarse network.

On Native plants the symptoms are somewhat different. The leaves



TEXT-FIG. 5. Transverse section of leaf affected by 'Leaf Roll', showing the collapse of the lower epidermis and the formation of the brown deposit.

are much crimped all round their margin, though the under surface is glazed and brown as in the American plant. Their appearance is reminiscent of the 'Curly Leaf' of Sea Island cotton in the West Indies (9). Plants affected seem to exhibit a remarkable tendency to shed their flower-buds.

*Histology.* A microscopic examination of affected leaves reveals a strange collapse of some of the lower epidermal cells (Text-fig. 5); these collapsed cells leave a thick brown deposit. Microchemical tests show that this deposit is by no means homogeneous. With Schultz's solution it stains predominantly yellow-brown with occasional flecks of a dark brown or violet colour; with Sudan III, used as suggested by Lee and Priestley (8), the whole mass is stained, indicating the presence of fatty substances. The pentose test (employing hydrochloric acid and phloroglucin) indicates the presence of pentoses at the corners of affected epidermal cells (Text-fig. 5, *a*), while suberin tests give an indefinite reaction. No abnormal features have been found in the petiole or other parts of the plant.

The cause of the downward roll of the leaf margin of American cotton is probably as follows: The substances replacing the cells of the lower epidermis are relatively inelastic. The upper epidermis, palisade, and spongy mesophyll, on the other hand, continue growing and expand against the

constraint of this inelastic layer. As a consequence, a downward roll is induced by the tissue tension thus set up. The Native cotton is less severely affected, and the rolling of the leaf margin is seldom encountered.

*Infectivity.* As will be shown in the subsequent section, diseased plants may recover and produce healthy leaves. When plants affected by 'Leaf Roll' are cut back, the new growth produced from dormant buds below the cut may be quite unaffected. Moreover, as the disease cannot be propagated by budding, it may, we think, be assumed that it is not infectious.

*Diurnal Gain and Loss in Dry Weight of Healthy and Diseased Leaves.* A comparison was made of the diurnal changes in the dry weight of diseased and healthy leaves. The results seem of sufficient interest to warrant their presentation (Table II). Errors caused by changes in volume due to incipient drying are probably negligible as the leaf discs were collected at dawn and dusk. Not less than 100 leaf discs, each approximately 7 sq. cm. in area, were employed in each determination.

TABLE II. *Showing Day-time Gain and Night-time Loss in Dry Weight in grm. per sq. metre of Leaf Surface.*

Number and Date of Experi- ment.	I. October 3-4.		II. October 11-13.			
	7 a.m.-6 p.m.	6 p.m.-7 a.m.	7 a.m.-6 p.m.	6 p.m.-7 a.m.	7 a.m.-6 p.m.	6 p.m.-7 a.m.
Healthy leaves.	+ 9.1	- 7.6	+ 10.5	- 10.5	+ 7.9	- 6.6
Diseased leaves.	+ 4.8	- 6.4	+ 3.0	- 3.9	+ 5.3	- 6.6

It will be seen that the increase in dry weight by day is consistently greater in healthy leaves than in those affected with 'Leaf Roll'. This might have been anticipated, but that the loss in weight during the night should exceed the day-time increment in diseased leaves is noteworthy. It would be unwise to stress this too much, since it is probably a feature that becomes marked only prior to the shedding of the leaf, for the leaves employed in the experiment were all severely affected.

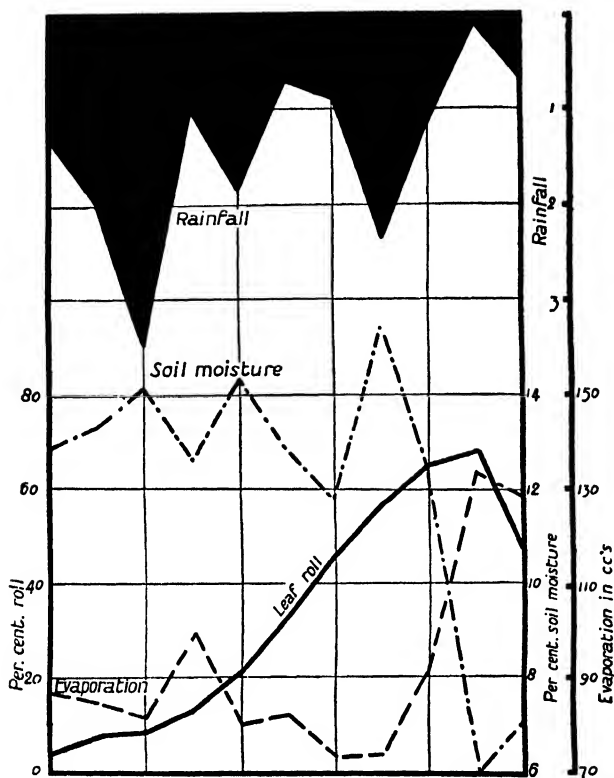
*Effect of Environment.* Four fields of American cotton were kept under observation. These fields were situated between the towns of Oyo and Ogbomosho and differed greatly in the character of their soils. It was on one of these fields that 'Leaf Roll' was first observed. A record was kept of the following:

(1) *The percentage of plants affected by 'Leaf Roll'.* A plant was judged to be affected if the under surface of the leaf presented a glazed appearance. About 3,000 plants on each field were examined weekly.

(2) *The percentage of soil moisture.* Soil samples were taken from each field twice a week and their moisture content determined. The results are expressed on the oven-dry basis.

(3) *Evaporation.* Livingston standardized white spherical atmometers were run in duplicate on two fields.

(4) *Rainfall.*



TEXT-FIG. 6. Showing mean percentage plants affected by 'Leaf Roll', soil moisture evaporation, and rainfall on four cotton fields between Oyo and Ogbomosho.

As the dynamic aspects of these characters did not differ markedly from field to field, the means for the four fields are presented graphically in Text-fig. 6. It will be seen that the number of plants affected increased until November 14. The soil moisture throughout this period only once dropped below 12 per cent. As the average hygroscopic coefficient of the soils was approximately 4 per cent., it will be evident that the water-supplying power of the soil was considerable. The moisture conditions became markedly drier in early November. It was then noticed that the leaves produced were no longer affected by 'Leaf Roll'. The reduction in the number of diseased plants which is shown in the figure on November 21

is presumably due to partial defoliation and the production of healthy leaves. The recovery of the plants on the onset of dry conditions suggests that one of the causative factors is excessive humidity.

The percentage of plants affected on the four fields ranged from 47 to 86, and the hygroscopic coefficients of the soils from 1.3 to 6.8, but as the hygroscopic coefficients of the fields with the highest and lowest percentages of diseased plants differed by only 0.2 it will be evident that soil texture is not a factor of any moment. The specific conductivities<sup>1</sup>  $\times 10^6$  at 0° C. of the 1 to 5 soil extracts varied between 18 and 44, but bore no relation to the incidence of the disease. The only factor other than excessive humidity that appeared to be associated with the disease was light. It was noticed that where there was much shade the plants seemed to be less affected. It should be added that Native cotton on neighbouring fields also recovered as the aridity increased.

*Experimental Production of 'Leaf Roll'.* An experiment was undertaken in early February, 1925, in order to determine whether the disease could be induced by excessive soil humidity and to what extent shade might operate as a factor inhibiting its appearance. The work was done in the dry season while evaporation ranged from 200 to 300 c.c. per week. Six batteries, each of 12 porous earthenware pots of approximately 60 litres capacity, were planted with American cotton. The pots, which were filled with a sandy loam, were sunk in the ground. Three batteries were heavily shaded with oil-palm leaves. Some difficulty was experienced in establishing the plants in these batteries and many had to be transplanted from the open. From 2 to 5 plants were grown in each pot. The treatment accorded each of the batteries is shown in Table III. Holes were made in the pots of batteries 3 and 6, and, as the soil in which the pots were embedded was very dry, there was no tendency to waterlogging. The specific conductivity  $\times 10^6$  at 0° C. of the water applied was 115.

The numbers of diseased and healthy plants were counted when the plants were about eleven weeks old. Diseased plants were divided into two classes. In the first were included plants with a glazed lower epidermis, and in the second those that also had the margin of the lamina rolled. The results exhibited in Table III seem to show that the water-supplying power of the soil *per se* is a factor of great importance. The absence of the disease from the shaded batteries is especially noteworthy. It must, however, be recollected that many of the plants in these batteries had been transplanted. The leaves in battery 6 presented an extremely turgid appearance, but were somewhat yellow. It would seem that, given adequate light and an abundance of soil moisture, the disease can be induced at will.

The nature of the internal factors that lead to a disorganization of the

<sup>1</sup> Mean of samples taken on Sept. 12, Oct. 10, and Nov. 7.

lower epidermis and a rolling of the leaf margin are quite unknown, but the external factors which are potent in inducing the disease suggest that possibly abnormal root pressures may be generated within the plant. The investigation will be carried farther when facilities for doing so are available.

TABLE III. *Showing Effect of Soil Humidity and Shade on the Production of 'Leaf Roll'.*

Number of Battery.	Treatment.	Number of Plants.		Normal.
		With glazed Lower Epidermis.	Glazed and rolled Leaf Margin.	
1	Shaded {	—	—	34
2		—	—	29
3		—	—	18
4	Not shaded {	—	1	39
5		10	10	23
6		24	17	8

#### SUMMARY.

The present paper contains an account of two rather obscure diseases of the cotton plant in Nigeria.

#### *Leaf Curl.*

1. The name 'Leaf Curl', proposed originally by Farquharson, has been retained for a disease which is especially prevalent on the indigenous species of cotton (*G. peruvianum*, *G. vitifolium*); it is found also on American cotton (*G. hirsutum*).

2. The histological modifications that characterize 'Leaf Curl' show a striking resemblance to the abnormalities that accompany virus diseases.

3. It has been found that the disease can be transmitted by budding. Infection has not been obtained by inoculation with the sap of diseased plants, nor by Jassids.

4. The incidence of 'Leaf Curl' is particularly pronounced in the spring.

#### *Leaf Roll.*

1. The term 'Leaf Roll' is suggested for a disease discovered on American cotton (*G. hirsutum*), on which it is more prevalent than on the indigenous species.

2. The under surface of leaves affected by 'Leaf Roll' present a glazed appearance. Microscopic examination shows that some of the cells of the lower epidermis are collapsed. The collapsed cells leave a thick brown deposit.

3. It has been shown that the disease is not infectious.

4. The most important predisposing environmental factor is excessive soil humidity *per se*, and not the indirect effects (e. g. root asphyxiation, leaching of solutes) that accompany an excessive quantity of soil water. The disease has not been noticed in plants growing under heavy shade.

5. Diseased plants recover and produce normal healthy foliage on the onset of dry conditions.

In conclusion we wish to record our indebtedness to our colleague, Mr. C. H. Wright, for placing his records of soil moistures, hygroscopic coefficients, and electrical conductivities of the soil extracts at our disposal.

We also wish to thank Professor S. F. Ashby and Dr. E. J. Butler for valuable help and criticism, and we are much indebted to Mr. Clyne for indefatigable assistance throughout the course of the work.

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EXPLANATION OF PLATE XXXII.

Illustrating Messrs. G. H. Jones and T. G. Mason's paper on Two Obscure Diseases of Cotton.

Fig. 1. The under surface of a healthy Native cotton (*G. peruvianum*) leaf.

Fig. 2. The under surface of a Native cotton (*G. peruvianum*) leaf affected by 'Leaf Curl', showing the upward curl of the leaf margins and the presence of the enations. (a) A small foliar outgrowth is indicated at A.

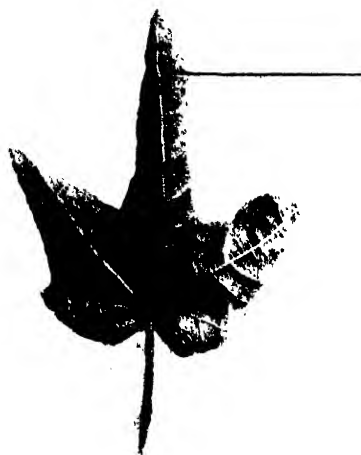
Fig. 3. The under surface of a healthy American cotton (*G. hirsutum*) leaf.

Fig. 4. The under surface of an American cotton (*G. hirsutum*) leaf affected by 'Leaf Roll', showing the downward roll of the leaf margins, the glazed under surface, and the fenestrations.





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# The Distribution of Certain Members of the British Flora.

## III. Irish and Anglo-Irish Plants.

BY

J. R. MATTHEWS, M.A.,

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With Five Diagrams in the Text.

### INTRODUCTORY.

OF the many remarkable features in the distribution of the flora of the British Isles, none has attracted more attention than the presence in the west of Ireland of a number of plants which have their continental headquarters in south-west Europe. These Hibernian species, or at least nine or ten of their number, constitute that small element in our flora which has been variously named Pyrenean, Lusitanian, or Cantabrian. Their isolation in Ireland presents a difficult problem in plant-geography, and more than one theory has been advanced to account for their peculiar range. By some authors the Pyrenean plants in west Ireland are regarded as relics of a preglacial flora which occupied a continuous land surface stretching from Ireland to Spain; others, finding it difficult to believe in survival throughout the whole period of the Pleistocene, consider the species to be post-glacial arrivals. If the weight of evidence be in favour of the survival hypothesis, the difficulty is not so much to account for the occurrence in the west of Ireland of a few species not elsewhere found in Britain, as to explain the history of a much larger number of species which form a definite southern element in our flora to which the Hibernian plants undoubtedly belong. This is an aspect of the problem which has been impressed upon the writer by a perusal of Dr. Stapf's papers (22, 23) on 'The Southern Element in the British Flora'. If the Pyrenean species, which reach in Ireland the northern limit of their present European range, are relics of a former widespread preglacial flora, one is led to inquire what proportion of the southern flora as a whole survived the glacial period in these islands. As Dr. Stapf

has shown, the Pyrenean species are members of a southern stock in the flora of Britain numbering in all about 150–160 species, and those few which are Hibernian cannot really be regarded as peculiarly isolated from the main area. Four of them, *Saxifraga Geum*, L., *S. umbrosa*, L., *Pinguicula grandiflora*, Lam., and *Erica Mackaii*, Hook., are often quoted as the most puzzling instances of distribution among British plants. The first three are found in west and south-west Ireland, and in the Pyrenees they reach their northern limit on the Continent; the last-mentioned occurs only in Galway and in northern Spain. But Dr. Stapf writes (23): 'The anomalies of their distribution are, however, more apparent than real, and considered side by side with the distribution of the other members of the southern stock, they resolve themselves into cases of far-gone disintegration of area. How this has come about or how the Atlantic and Mediterranean elements of the British flora have arrived in their island home is a question which cannot be dealt with in this place.' 'This southern element,' to quote Dr. Stapf again (22), 'is like a weft in a woven fabric. It has not come alone. It is associated here in these islands with species which we call Central European or Germanic, although they are also found in the Pyrenees and the mountains of northern Spain. At whatever period this element may have come into Great Britain and Ireland we must not think of its constituents as wandering singly and independently of each other.'

The maps which accompany Stapf's second paper show that this southern element, advancing from south-west Europe, has established itself chiefly in our south and south-west counties, thence spreading northwards, especially along the west coast. The group as a whole shows a remarkable continuity of the area occupied, and although the English and Irish Channels cut into the area, they do not interrupt the natural trend of these southern species forming a belt which skirts the Bay of Biscay and the English and Irish Channels. The mass distribution provides evidence of a general movement from the south-west. Dr. Stapf does not discuss the period when these southern species may have reached Britain, but in his introductory remarks he states: 'Whether one accepts the "land-ice" or the "submergence" theory, both of which have been dealt with so admirably by Professor Bonney, the botanist cannot but assume that survival under the rigorous conditions postulated by both theories was impossible for most or probably all the plants under consideration. If in the future new facts should come to light which make the climatic conditions during the glacial period appear more favourable for plant life, the question of survival will have to be reconsidered.'

The 'southern element' constitutes only 9 per cent. of the British flora, and in Europe has its head-quarters in the west or south. The bulk of our flora, however, has a wide range on the Continent, and other migrations from the mainland must have occurred. In previous papers of this

series (10, 11) attempts were made to assemble the data which would provide some definite evidence regarding the direction of plant immigrations from the Continent. The 'English' flora proved especially suitable, and on analysis it appeared as a composite flora in its derivation from the European mainland by separate invasions coming from different directions. Four lines of dispersal were indicated. One of these, which may be viewed as belonging to the same general drift suggested by Stapf's cartographic study, seems to have resulted in a concentration of species in the Peninsula province of England, providing a flora which exhibits a coastal tendency as far as Norfolk in the east and Westmorland in the west, but thinning out rapidly inland. Such an invasion might be expected to reach the shores of Ireland, thus to play a part in the building up of the Irish flora. That this has happened seems a reasonable conclusion from an analysis of that portion of our flora which is confined to Ireland, England, and Wales. On the other hand, a similar invasion carried a few degrees to the west would reach Ireland without touching England, and so account for species confined to Ireland. These two groups of plants—Irish and Anglo-Irish—are dealt with in the following pages, their distribution being presented cartographically along the lines adopted in my previous papers.

*Distribution of Sixteen Species restricted to Ireland.*

The following sixteen flowering plants are not known as native within the British Isles, except in Ireland :

<i>Arabis ciliata</i> , Br.	† <i>Daboccea polifolia</i> , Don.
<i>Arenaria ciliata</i> , L.	<i>Euphrasia salisburgensis</i> , Funk.
† <i>Saxifraga umbrosa</i> , L.	† <i>Pinguicula grandiflora</i> , Lam.
† <i>Saxifraga Geum</i> , L.	† <i>Habenaria intacta</i> , Benth.
<i>Inula salicina</i> , L.	* <i>Spiranthes Romanzoffiana</i> , Cham.
† <i>Arbutus Unedo</i> , L.	* <i>Sisyrinchium angustifolium</i> , Mill.
† <i>Erica Mackaui</i> , Hook.	<i>Potamogeton Kirkii</i> , Syme.
† <i>Erica mediterranea</i> , L.	† <i>Glyceria festuciformis</i> , Heyn.

From this list of Irish plants *Helianthemum guttatum*, Mill., is excluded on the ground that the type, which occurs only in Ireland, is linked by its variety *Breweri* to an English station in Anglesey and to a wide south-west continental range north to Holland by its stations in the Channel Isles. There has been added to the list *Arabis ciliata*, an endemic species, which appears to be confined to Ireland, the Welsh plant long referred to this species having been shown by Salmon (19) to belong to *A. hirsuta*. Taxonomic and distributional details have been given for the arctic *Arenaria ciliata* by Ostenfeld and Dahl (13), who refer the Irish form to sub-sp. *hibernica*. Two species marked with an asterisk are American, and are

excluded from the distribution map shown in Diagram 1, although *Sisyrinchium angustifolium* has been reported as adventitious in Germany and in Norway. It is regarded as possibly introduced in west Ireland. The case is different for the American *Spiranthes Romanzoffiana*, which is accepted as native in its Irish stations. Of the remaining species named in the list, *Inula salicina* and *Euphrasia salisburgensis* are noteworthy outliers from their continental range. Both are widely distributed in Europe and both extend into Asia. Their absence from England is remarkable. *Potamogeton Kirkii* is regarded by Hagström (7) as a hybrid between *P. gramineus*, L., and *P. natans*, L., and is referred to *P. sparganifolius*, Laest. It is known also from Norway, Sweden, and North America.

There remain nine species marked with a dagger which may be described generally as the Iberian element that enters into the composition of the Irish flora. They have their chief centre in northern Spain, but several extend eastwards into the Mediterranean area and a few range northwards into France. Dr. Stapf (22) gives distributional details regarding most of these species, and illustrates the progressive series of gaps which exist between their stations in Ireland and the nearest continental areas.

Diagram 1 shows the distribution of the Irish group of species excluding those two which are American. The data have been taken from Lloyd Praeger's 'Irish Topographical Botany' (14) and the same author's Supplement published in 1906. In the inset map the European range is indicated as determined from Nyman's 'Conspectus Florae Europaeae', and from numerous continental floras. While the Hibernian species are essentially western, it is of interest to note that the Mediterranean *Glyceria festuiformis*, a maritime plant, has established itself in north-east Ireland. Doubts have been expressed regarding its indigeneity, but Dr. Praeger, who discovered the plant in 1903, regards it as native in its Irish stations.

Disintegration of area may explain the present restricted range of certain of these Hibernian plants, yet the distribution of a number of species common to Ireland and England points to a floristic connexion between the two countries and with the Continent. These are the Anglo-Irish species, numbering sixty-eight.

#### *Distribution of Sixty-eight Anglo-Irish Species.*

It is worthy of remark that certain plants widely distributed in England and in Ireland should have failed to reach Scotland. To plant dispersal there are many barriers, and climatic factors may prevent an extension northwards of many of the Anglo-Irish species. Yet fourteen of them reach Norway and a few might be expected to occur in Scotland. Water plants frequently exhibit a wide range, and in the list of sixty-eight species there are as many as ten aquatics. Among them are *Myriophyllum verticil-*

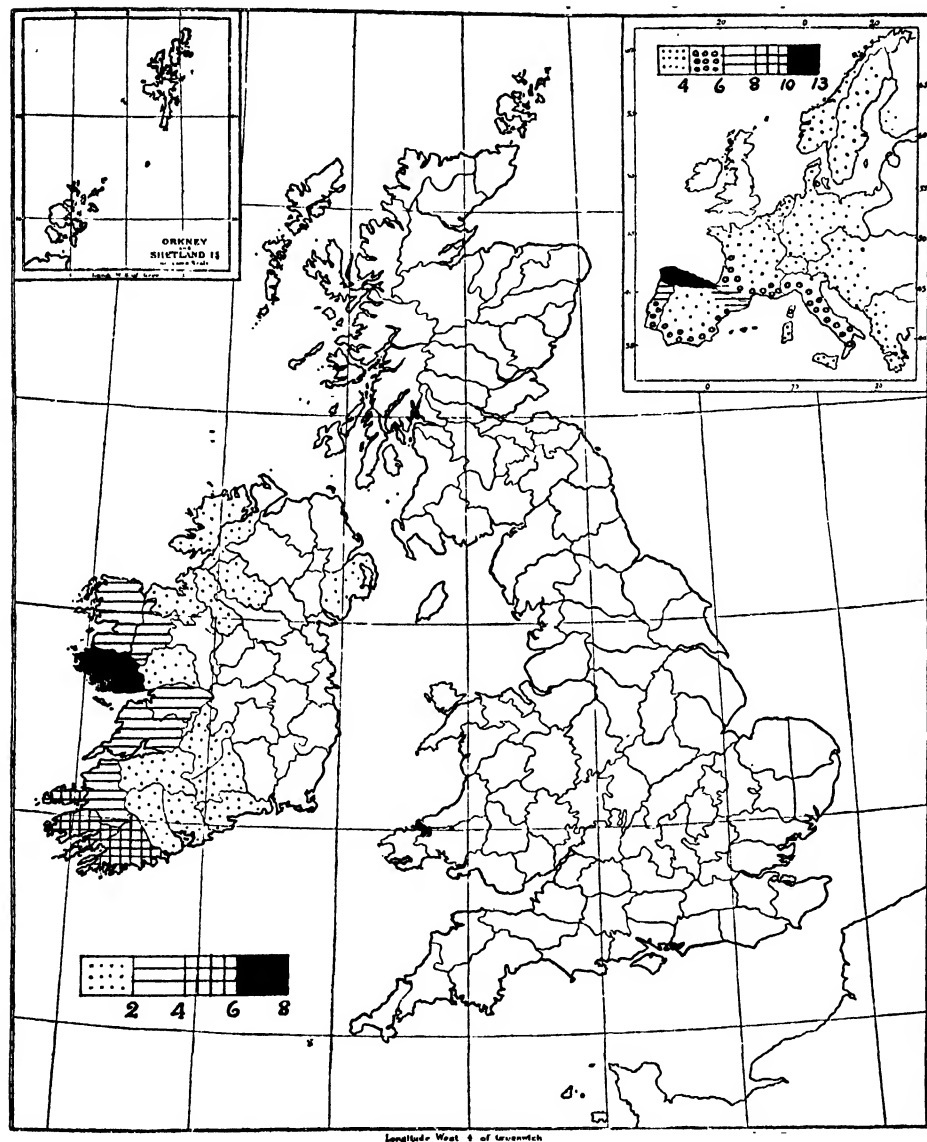


DIAGRAM 1. Distribution of fourteen species confined to Ireland.

*latum*, L., *Callitriche obtusangula*, Le Gall., and *Sagittaria sagittifolia*, L.—three species which are fairly widely distributed in England and in Ireland, but they do not occur as natural constituents of the flora north of the Cheviots. Although the Arrowhead has been recorded from south-west Scotland it cannot be claimed as certainly native in its Scottish localities.

The distribution of the Anglo-Irish species is shown in Diagram 2; the inset map indicates the range of the group on the Continent. The map is constructed from the topographical statistics for Great Britain given by Watson (26) and by Bennett (1). The data for Ireland are taken, as for Diagram 1, from Lloyd Praeger's works on Irish Topographical Botany. A few species are doubtfully native in Ireland. Those regarded by Praeger as certainly introduced have been excluded. *Arabis ciliata*, Br., long regarded as a species peculiar to Ireland and Wales, is transferred to the Irish list for reasons already given.

The map (Diagram 2) shows a concentration of species in the south and south-east counties of England with an extension into Somerset and west Gloucester. In these two areas and in each of the coastal vice-counties from south Devon to Norfolk, except south Essex, at least 50 per cent. of the total number of species are found. Dorset and east Kent stand highest with about 68 per cent. From the region of density in the south and south-east a fairly steady decrease can be traced towards the north and north-west. The low figure for north Hampshire is peculiar, while Carnarvon, Anglesey, and mid-west York have rather high figures. The mass distribution of these 'Anglo-Irish' species within England and Wales is not dissimilar to that of the entirely 'English' group of species shown in Diagram 1 in my former paper (10). The same general drift can be detected. Advancing through France, it passes across England from south-east to north-west. But in the present assemblage the extension is greater. For among the 'English' species only 30-40 per cent. are concentrated in the south-east counties, while of the 'Anglo-Irish' species at least 30 per cent. are found over the greater part of England, with over 50 per cent. in the south-east. There is, moreover, a noteworthy feature in the distribution of the population now under consideration in its greater tendency towards the south-west, a feature which characterizes also its continental range. Of the sixty-eight species, twenty are members of the southern element as defined by Stapf, six of these being Atlantic. With two exceptions, *Allium Babingtonii*, Borr. (endemic in Britain) and *Potamogeton varians*, Fryer, all occur in France, and over 75 per cent. in Spain and in Italy, while about 65 per cent. are recorded from central Europe. There seems little doubt, then, that this 'Anglo-Irish' flora in England has been derived from the mainland along migratory paths whose directions have not been essentially different from those followed by our 'English' flora as previously suggested. But the dominant, though not the only, migration has been one from the



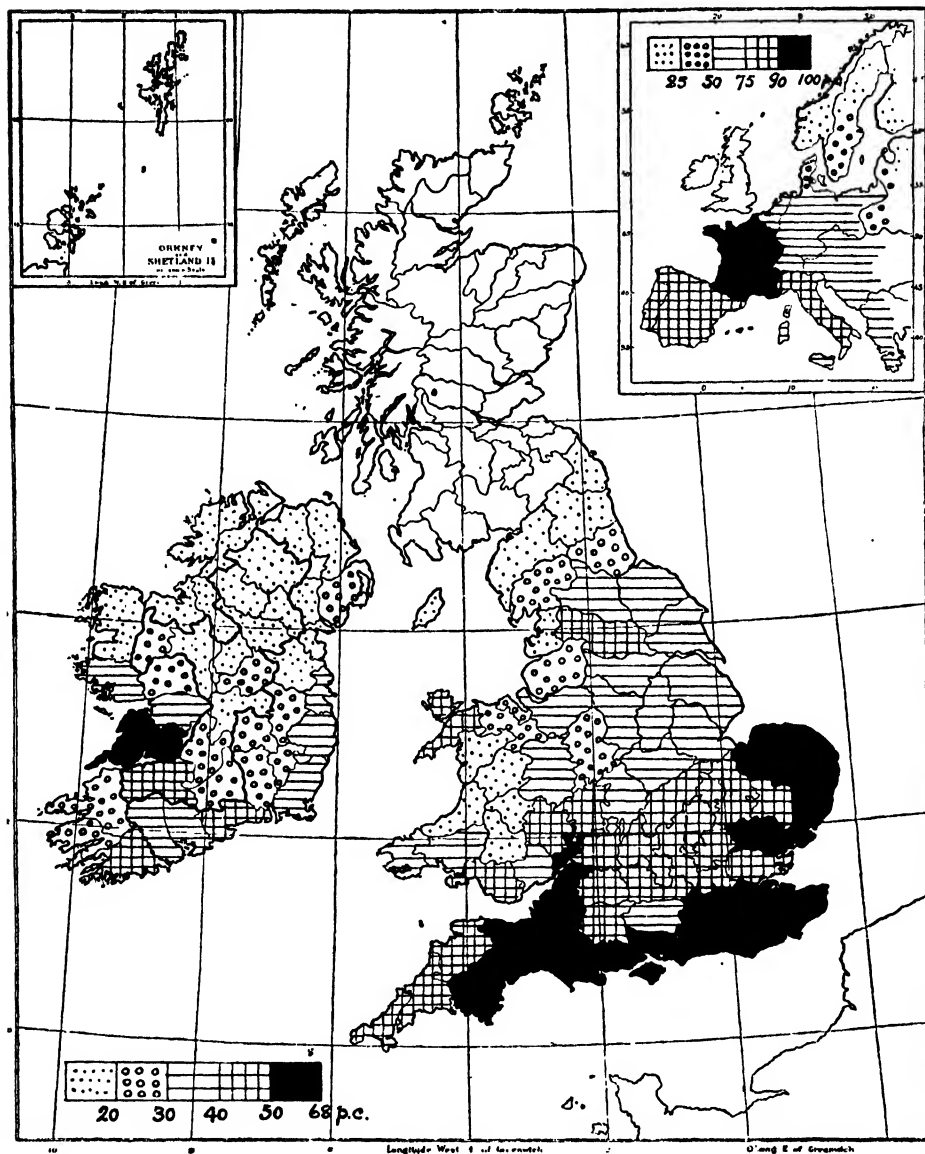


DIAGRAM 2. Distribution of sixty-eight species confined to Ireland, England, and Wales.

south or south-west, related, in all probability, to the general prevalence of the group in France and south-west Europe. The broad outlines of dispersal within England, pointing to such a movement, are more clearly indicated, perhaps, when the figures for Watsonian provinces are considered rather than the much smaller vice-counties. The largest number of species, 57 out of 68, is found in the Peninsula. If the provinces round the south and east be taken in sequence, the figures for Channel, Thames, Ouse, and Trent areas are found to be 52, 50, 47, and 33 respectively. The Severn province, which is mainly inland, has 42, South Wales 38, and North Wales 44 species. Such findings illustrate the western tendency of this limited flora, and to this tendency must be attributed its presence in Ireland.

But within Ireland certain anomalies appear. Mid and east Cork, with relatively low figures, are bordered by Waterford, Limerick, and west Cork, each showing a greater density, while Clare in the west is the only division in the island with 50 per cent. of the total. This uneven distribution is somewhat unexpected, and one line of inquiry would be a study of the distribution and ecology of individual species. The fact remains, however, that in the southern half of the island (divisions 1-20) all but three of the sixty-eight species are found, while forty-three occur in the northern half (divisions 21-40), a dispersal which indicates a south to north movement. Moreover, the movement has been chiefly a marginal one, for both in the east and in the west of the island there are fifty-three species, while the centre (marked in the map by a heavy line) possesses forty species. The existence of as many species in the west as in the east is a point of interest to which further reference will be made, since there are several members of the Anglo-Irish group whose appearance in the west of Ireland cannot readily be explained by reference to their distribution in England or on the European mainland.

*Distribution of the Rarer Anglo-Irish Species.*

From this general survey we may pass to an analysis of the rarity of the Anglo-Irish plants. Arranging them in five classes according to the number of Watsonian vice-counties and Irish divisions occupied, we obtain the figures given in Table I.

TABLE I.

<i>England and Wales.</i>			<i>Ireland.</i>		
<i>Occupying not more than—</i>		<i>Number of Species.</i>	<i>Occupying not more than—</i>		<i>Number of Species.</i>
Fourteen vice-counties . . .	.	27	Eight divisions . . . . .	.	42
Twenty-eight vice-counties . . .	.	9	Sixteen " . . . . .	.	10
Forty-two " . . . . .	.	12	Twenty-four divisions . . . . .	.	7
Fifty-six " . . . . .	.	13	Thirty-two " . . . . .	.	7
Seventy-one " . . . . .	.	7	Forty " . . . . .	.	2

The average size of the 71 Watsonian vice-counties and of the 40 Irish divisions is approximately the same, the figures being 821 and 815 square miles respectively. It is, therefore, of interest to note that while 27 species occur in not more than 14 English vice-counties as many as 52 are found in not more than 16 Irish divisions. The degree of rarity in Ireland is high, the species occupying, on the average, 27·6 vice-counties in England and Wales, and 9·9 divisions in Ireland. In terms of the 'Age and Area' law propounded by Dr. Willis (27) the more limited dispersal in Ireland would be explained by more recent arrival. In the average case we should expect this, since Ireland is farther from the continental source of supply, and England rather than the Continent may have been the centre of dispersal into Ireland. While this may have been the general course of events, the distribution of certain individual species suggests that it has not been followed invariably.

While the figures in Table I, which refer to Ireland, may point to a flora at a relatively early stage of spreading, it is different in the case of those which relate to the same flora in England. Yet the actual distribution within England and Wales, as mapped in Diagram 2, is indicative of a fairly definite movement. But it has been a wide, diffuse movement, and is in reality the resultant of several invasions coming from different directions. The ultimate commingling of species gives rise to a plant population the numeral analysis of which, taken as a whole, may show little evidence in support of the 'Age-and-Area' rule. Each invasion may follow the law, but the difficulty is to disentangle the invasions. In the course of time, as invasions converge, a concentration of the flora in the area will follow. The more recent the invasion the more likely is the working of the 'Age and Area' principle to be detected.

If, then, the Anglo-Irish species can be regarded generally as a relatively recent portion of our flora, we should seek evidence of its migratory paths among those members that are still somewhat restricted in their range. Diagram 3 shows the distribution of those thirty-six species which in England and Wales occur in not more than twenty-eight vice-counties. They are relatively rare species. In Ireland as many as thirty-one of them are found in not more than eight divisions. Details are given in Table II.

TABLE II.

<i>England and Wales.</i>				<i>Ireland.</i>			
<i>Occupying not more than—</i>		<i>Number of Species.</i>		<i>Occupying not more than—</i>		<i>Number of Species.</i>	
Seven vice-counties	.	.	16	Two divisions	.	.	19
Fourteen vice-counties	.	.	11	Four "	.	.	5
Twenty-one "	.	.	5	Six "	.	.	6
Twenty-eight "	.	.	4	Eight "	.	.	1
				Twenty-six divisions	.	.	5

An examination of Diagram 3 shows that these rarer species are most frequent in a number of the south and west coastal counties. East Kent has twelve species, but the main area lies farther west, embracing Isle of Wight, south Hants, Dorset, and the whole Peninsula province (except south Somerset) in England, and Glamorgan, Pembroke, and Anglesey in Wales. The coastal tendency is partly explained by the fact that nine species are littoral. The figures for the midland counties are low; those for Yorkshire higher than might be expected; but two species, *Potentilla fruticosa*, L., and *Gentiana verna*, L., are boreal. The numbers for Watsonian provinces are also given. The Peninsula area stands highest with 25 species; the Channel, Thames, Ouse, and Trent have 20, 18, 15, and 3 respectively. From South Wales 15 of the 36 species are recorded, and 14 from North Wales. The Humber province is rather high with 9 species.<sup>1</sup>

Diagram 3 shows, also, the range of these 36 species in Ireland. Only in 4 divisions are there at least 10 species as against 12 vice-counties in England and Wales. County Clare stands highest with 14, two of these being the boreals already mentioned, and in west Cork, Waterford, and Wexford at least 10 species occur. In the southern half of the island there are 34 species, while from the northern half 18 are recorded. *Elatine Hydropiper*, L. (in the north-east), and *Carex paradoxa*, Willd. (north-centre), are the two species absent from the south. From the west portion of the island 26 species are recorded, 22 from the east, and 14 from the centre. To make possible a closer comparison with the Watsonian provinces of England and Wales, east and west Ireland have each been subdivided into three (see brackets in map) and the centre into two districts. It is then found that the figures for south-east, mid-cast, and north-east districts are 14, 14, and 9 respectively; for south-centre and north-centre, 10 and 8; and for south-west, mid-west, and north-west 14, 16, and 9 respectively. These facts suggest a migration from the south whereby plants have reached and established themselves in Ireland chiefly in that coastal strip from Cork to Wexford, an area which lies roughly parallel to the Peninsula province of England, where, as we have seen, most of the rarer species occur. The outstanding anomaly is County Clare. Moreover, the group is essentially south-western in Europe. All but the endemic *Allium Babingtonii*, Borr., occur in France, and thirty occur in Italy and in Spain.

The analysis of the rarer species is carried a step farther in Diagram 4, which shows the distribution of the twenty-five members found in the Peninsula province of England. Comparison should be made with Dia-

<sup>1</sup> Of the eleven rarer species not reported from the Peninsula, five occur in the neighbouring provinces, Channel, Severn, or South Wales. Of the remaining six, *Helianthemum guttatum*, Mill., is confined to Anglesey; *Potentilla fruticosa*, L., and *Gentiana verna*, L., are boreal; *Viola stagnina*, Kit., is eastern (Ouse and Trent), as is also *Carex paradoxa*, Willd. (Thames, Ouse, Trent, and Humber); *Potamogeton lanceolatus*, Sm., a hybrid pondweed, occurs in the Ouse province and in North Wales.

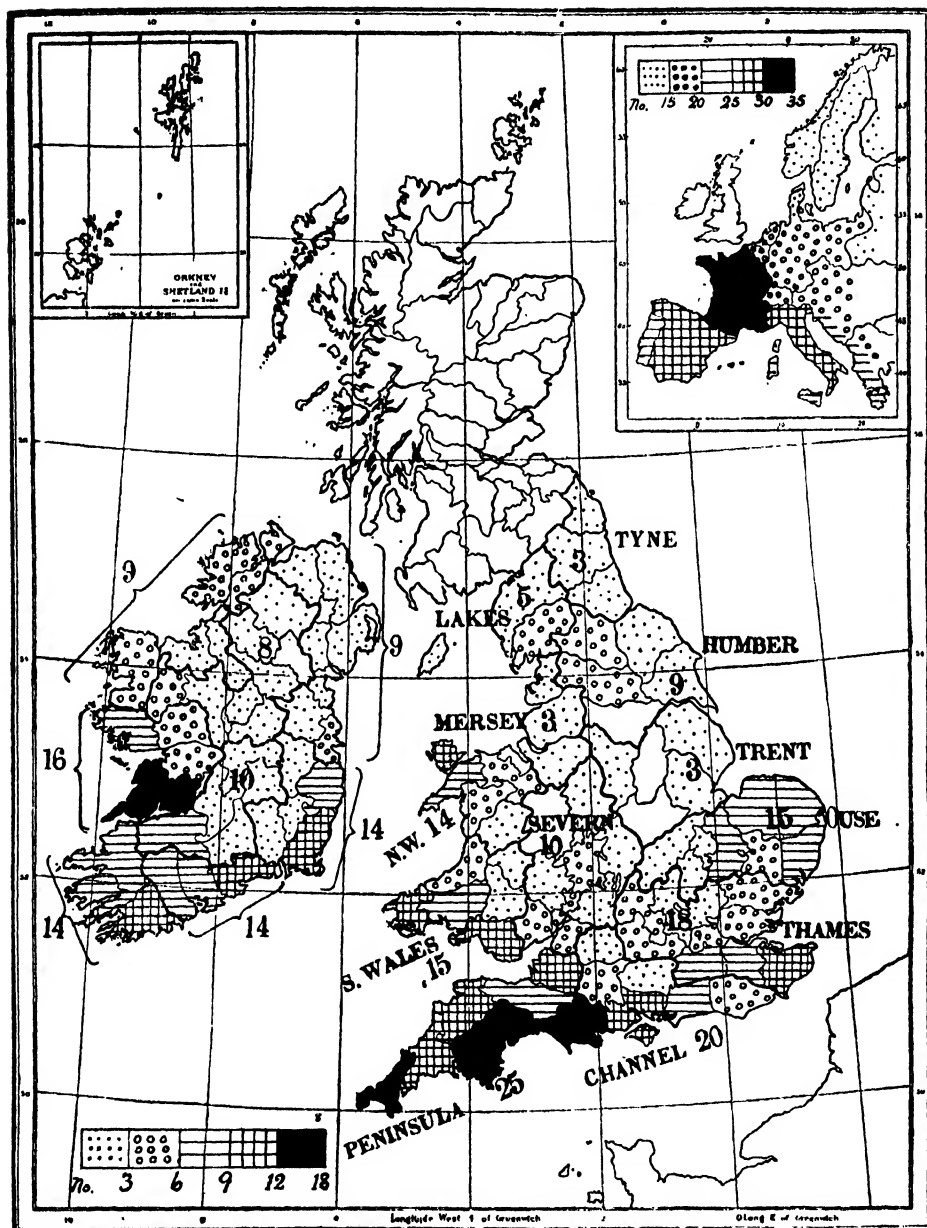


DIAGRAM 3. Distribution of thirty-six rare 'Anglo-Irish' species.

gram 3. These Peninsular species have their European head-quarters in the south-west; eleven extend into west Germany. It is therefore necessary, even in this small group, to allow for the arrival of species in south-east England from central Europe, and in this connexion the occurrence of as many as twelve species in east Kent may be noted. But the assemblage, as a whole, must be viewed as a Peninsular one which, in these islands, has disseminated outwards from that area as its main centre.

The chief interest of this Peninsular group, however, lies in its relationships within Ireland. An examination of the data shows that it accounts for the majority of the rarer species in the several districts of the island except the north-centre and mid-west. Thus, in the south-centre the ten rarer species are all Peninsular, all but one in the south, mid, and north-east districts, and all but two in the south-west. We find, therefore, in the geographical range of the rarer Anglo-Irish species a close connexion between the two countries. The link between south Ireland and the Continent is the Peninsula province in England. The migration to these islands which has established this phytogeographical connexion may be called the Peninsular invasion.

*Distribution of Anglo-Irish Species as determined by Range in Ireland.*

The prevailing western tendency of the Anglo-Irish species in Ireland (marked by the density in County Clare) is a feature which characterizes the distribution not only of the whole group (Diagram 2), but also the rarer species (Diagram 3). This feature one would be unlikely to predict from a knowledge of the range of the plants in England, and it suggested an examination of the species from the standpoint of their distribution within Ireland. The sixty-eight species have been classified in terms of the 'type of distribution' they present in Ireland. These 'types' have been defined and illustrated by Lloyd Praeger (15), and his classification is here adopted. Seventeen species are generally distributed over the island, but fifty-one show some special feature in their range.

These are grouped as follows:

<i>Type of Distribution.</i>	<i>Number of Species.</i>
Central	8
Marginal	1
Ultonian	1
Mumonian	23
Lagenian	6
Connacian	12

The species of the Central group are:

<i>Myriophyllum verticillatum</i> , L.	E.	<i>Ophrys apifera</i> , Huds.	EG.
<i>Crepis taraxacifolia</i> , Thuill.	G.	<i>O. muscifera</i> , Huds.	EG.
<i>Teucrium Scordium</i> , L.	GE.	<i>Sagittaria sagittifolia</i> , L.	E.
<i>Orchis Morio</i> , L.	E.	<i>Carex paradoxa</i> , Willd.	LI.

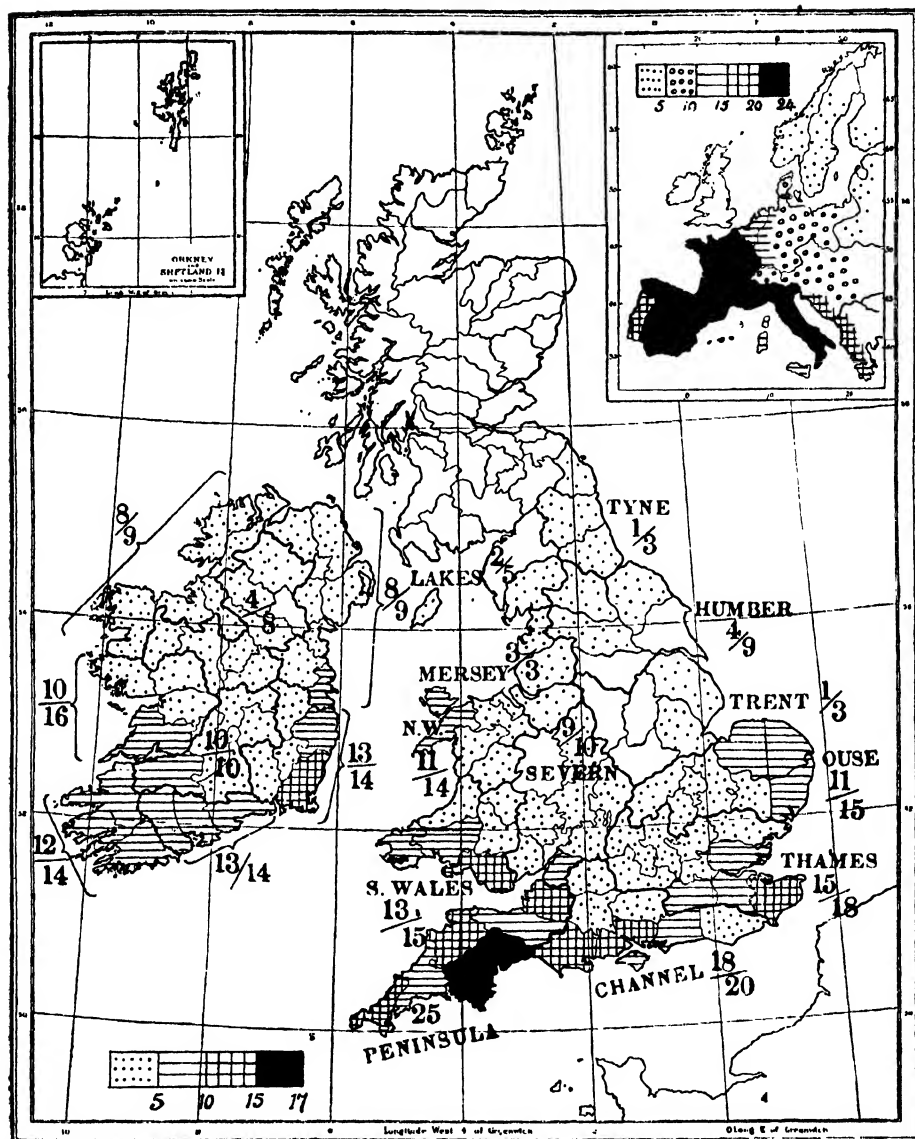


DIAGRAM 4. Distribution of twenty-five rare 'Peninsula' species of 'Anglo-Irish' group.

Within Ireland these eight species occupy, on the average, 16·6 divisions; in England and Wales forty vice-counties. It will be seen from the letters placed after the names, which refer to Watson's types of distribution in Britain, that three are generally distributed in England, four have a Germanic tendency, and one is local. But all seem to favour the central plain of Ireland, three of them, doubtless, because of their calcicole preferences.

The only Marginal species of our group is *Erodium moschatum*, L'Hér., an Atlantic plant in England occurring in fourteen counties from Dorset to Anglesey and Isle of Man. In Ireland it ranges over twenty divisions, mainly coastal.

The Ultonian type is *Polygonum mite*, Schrank. It occurs in four northern Irish divisions and reappears in the south in Limerick. Reported from twenty-four English vice-counties, mainly southern and eastern, it extends as far north as Yorkshire. It is absent from Wales.

The Mumonian or more southern species constitute the majority. The list of twenty-three 'Anglo-Irish' members is as follows:

<i>Ranunculus tripartitus</i> , DC.	EL.	<i>Sibthorpia europaea</i> , L.	A.
<i>Matthiola sinuata</i> , Br.	A.	<i>Calamintha officinalis</i> , Moench.	E.
<i>Lepidium latifolium</i> , L.	E.	<i>Verbena officinalis</i> , L.	E.
<i>Linum angustifolium</i> , Huds.	AE.	<i>Rumex pulcher</i> , L.	E.
<i>Geranium rotundifolium</i> , L.	E.	<i>Spiranthes autumnalis</i> , Rich.	E.
<i>Oenanthe pimpinelloides</i> , L.	E.	<i>Colchicum autumnale</i> , L.	E.
<i>Rubia peregrina</i> , L.	A.	<i>Juncus acutus</i> , L.	EA.
<i>Diotis maritima</i> , Cass.	A.	<i>Scirpus parvulus</i> , R. and S.	—
<i>Chlora perfoliata</i> , L.	E.	<i>Carex divulsa</i> , Stokes.	EG.
<i>Cicendia pusilla</i> , Griseb.	EA.	<i>C. axillaris</i> , Good.	E.
<i>Antirrhinum Orantium</i> , L.	E.	<i>Brachypodium pinnatum</i> , Beauv.	GE.
<i>Linaria Elatine</i> , Mill.	E.		

The average range of these species in England and Wales is 31·4 vice-counties, while for Ireland the figure is 8·3. Twelve species exhibit an 'English' type of distribution according to Watson's scheme, while seven have or show an Atlantic tendency. Only two are on the Germanic side. But a better idea of the range of this group is obtained from the map shown in Diagram 5. The group, as has been said, is determined by its distribution in Ireland, where it is southern, the main area extending from west Cork to Wexford. It has a wide range in west and south Europe and over 50 per cent. of its members occur in central Europe. It is an assemblage which illustrates very well that admixture of southern and central European species which exists within the British Isles. While a certain unevenness of distribution is manifest in England among the small vice-counties, the incidence of species in the larger provinces gives some indication of the



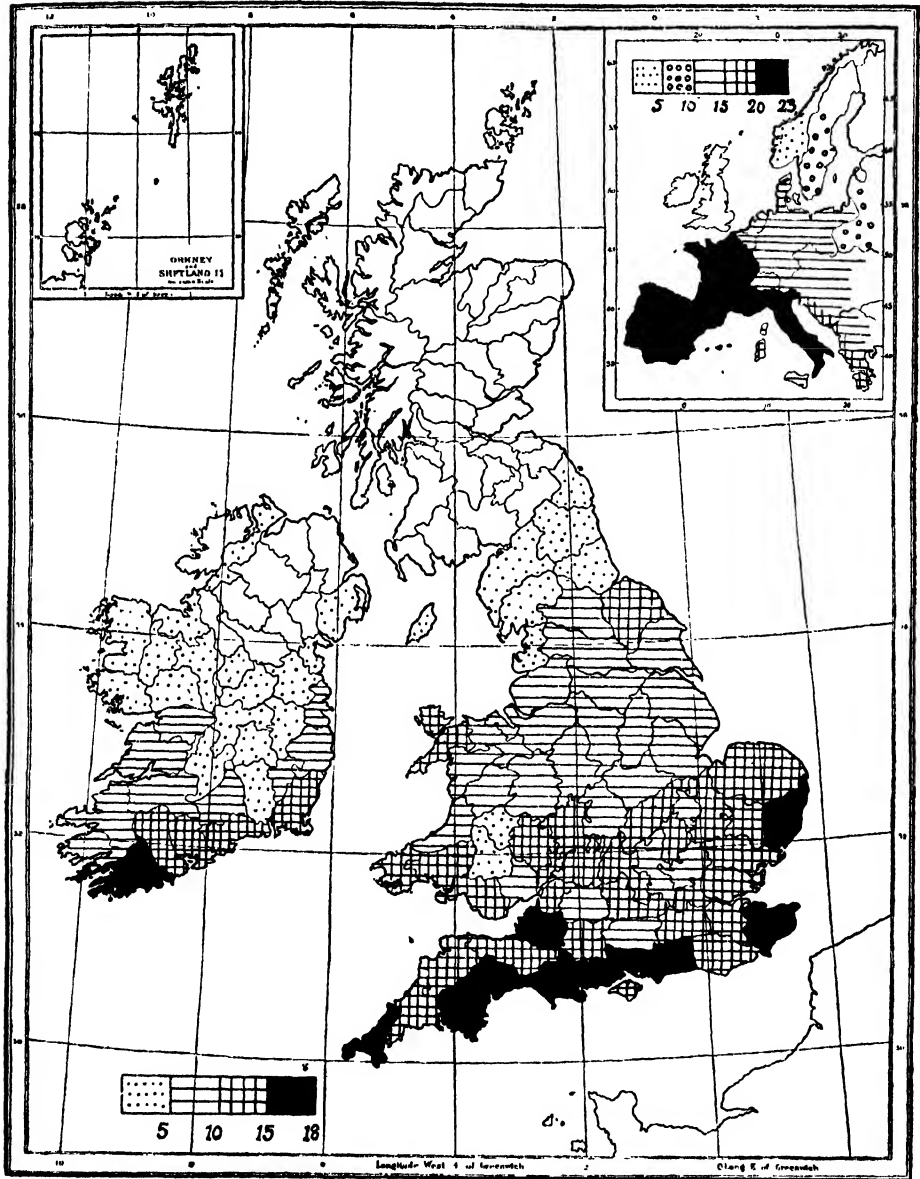


DIAGRAM 5. Distribution of twenty-three 'Mumonian' species of 'Anglo-Irish' group.

prevailing line of spread. For all are found in the Peninsula, twenty occur in the Channel, eighteen in the Thames, seventeen in the Ouse, and nine in the Trent province. From the Severn and from South Wales fourteen members of the group are recorded. A migration from the south, having a western tendency, would explain these leading features. The group, as a whole, seems older in England than in Ireland, and, if it has reached Ireland via England, the connexion would appear to be mainly through the Peninsula and South Wales provinces.

The Lagenian or Eastern species of Ireland among Anglo-Irish plants are the following :

<i>Elatine Hydropiper</i> , L.	EL.	<i>Asparagus officinalis</i> , L.	A.
<i>Trifolium subterraneum</i> , L.	E.	<i>Glyceria Borreri</i> , Bab.	G.
<i>T. glomeratum</i> , L.	E.	<i>Festuca uniglumis</i> , Sol.	EA.

Again, these are more widely distributed in England than in Ireland, the average range being 17·3 vice-counties and 2·3 divisions respectively. In Ireland they extend from Waterford to County Down, with four species in Wexford. For three of them the south-east counties in England are their main area ; two are western and *Elatine* is local.

The plants of the west of Ireland present a more puzzling assemblage. Dr. Praeger gives a list of fifty-five phanerogams which show the Connacian type of distribution. The areas of greatest density are south Kerry, Clare, and west Galway. Eleven members of the group are not found in Britain, and sixteen may be regarded as boreal or arctic-alpine. Twelve species belong to the restricted Anglo-Irish flora we have been considering. They are :

<i>Helianthemum canum</i> , Baumg.	IA.	* <i>Euphorbia hiberna</i> , L.	LA.
* <i>H. guttatum</i> , Mill.	LA.	<i>E. amygdaloides</i> , L.	E.
<i>Viola stagnina</i> , Kit.	EG.	* <i>Simethis bicolor</i> , Kunth.	LA.
† <i>Potentilla fruticosa</i> , L.	IS.	<i>Potamogeton lanceolatus</i> , Sm.	L.
<i>Asperula cynanchica</i> , L.	E.	<i>Allium Babingtonii</i> , Borr.	—
† <i>Gentiana verna</i> , L.	I.	<i>Scirpus triquetus</i> , L.	G.

Even this small group is a heterogeneous one. Two marked † are boreal, and three marked \* are southern, being absent from central Europe. *Allium Babingtonii* is endemic, and *Potamogeton lanceolatus* is of hybrid origin, having a very limited distribution both on the Continent and in our own islands. There remain five species widely distributed in Europe except in the north. Of these, *Viola stagnina* has the widest range, although in England it is restricted to the Ouse and Trent provinces. *Asperula cynanchica* is general in England except the north-east. *Euphorbia amygdaloides*, widely dispersed in England, is probably native in Ireland only in mid and west Cork, but reappears in Donegal. The main centre for

*Scirpus triquetus* is the Thames province, but it occurs in the Peninsula, and in Ireland it is found in Limerick and Clare. *Helianthemum canum* has its main area in Wales with extensions into Yorkshire and Westmorland, and appears in Clare and west Galway.

While this small group is confined almost entirely to the west of Ireland, it is scattered sparsely over England, and it is difficult to correlate the topographical data. The species illustrate varying degrees of discontinuity of area within these islands, and until they are studied individually it is not easy to suggest how this may have arisen. No general conclusion can be based on so small a number of species; yet, given their geographical range in England and in Europe and a knowledge of their occurrence in Ireland, one would scarcely have predicted their absence from the east of that island. Lloyd Praeger (15), writing of the Irish flora, draws attention to the wealth and variety of the flora of County Clare. In that county, 'where the calcicole flora attains its greatest development, the calcifuge flora is also at high-water mark; but in the rich and remarkable flora of that varied county, almost every group, whether English or Scottish, Atlantic or Germanic, calcicole or calcifuge, attains or approaches its maximum!'

#### DISCUSSION.

The following remarks are based upon this and my two previous papers. I introduce, also, some notes regarding the flora of the Channel Isles, which, geographically, belong to continental Europe, but certain features in the floristic composition of these islands throw an interesting sidelight on the history of our own flora.

In 'Island Life' Alfred Russel Wallace (25) expresses the opinion that the facts which he brings together respecting the peculiarities of the British fauna and flora 'are sufficient to show that there is considerable scope for the study of geographical distribution even in so apparently unpromising a field as one of the most recent of Continental islands'. Much detail has been added to our knowledge of distributional data since these words were written, but the main facts remain the same, and many phytogeographical problems await solution. Still open, for example, is the question whether the origin of an island flora is to be related mainly to slow overland migration before separation from the continental area, or to chance methods of dispersal over the sea. This has been the subject of much debate in connexion with the origin of our own flora, and certain outstanding groups, such as the Pyrenean and arctic-alpine elements, have made a special appeal. But the questions which arise will be dealt with satisfactorily only when every debatable species is examined critically with due consideration given to its continental as well as its insular range, and to the conditions of soil and climate which play a part in determining that range. Even then

the analysis will not be complete, for we shall have still to reckon with the organic environment and that competition among living organisms for establishment in areas which, from one cause or another, become available for colonization.

In presenting a summary of the views to which I have been led by these distribution studies, I may be allowed to recall that the studies commenced with the aid of the 'Age and Area' hypothesis of Dr. Willis, since that hypothesis suggested a new viewpoint in the field of plant-geography, particularly in relation to plant migrations. To discover invasions has been my chief aim, and to do so a cartographic presentation of the facts of distribution became necessary. From the maps which have been constructed it has become possible to trace approximately several, if not all, of the invasions that have contributed towards the building of our indigenous flora. Portions of the flora which show a restricted range within the British Isles have been analysed, since only those species which are limited to certain areas are likely to provide a clue to the direction whence they came.

That any lines of dispersal can be traced at all depends upon the fact that our flora is largely an invasion flora. Presumably it replaced, in whole or in part, a pre-existing vegetation. This aspect of an invading flora is sometimes overlooked, yet the replacement on a part of the earth's surface of one flora by another presupposes some underlying cause which favours the change and makes it possible. In Britain, it has been maintained by certain writers, notably Clement Reid (17), that the severity of climatic conditions during the glacial epoch was such as to leave the country practically devoid of vegetation, and the incoming of the present plant population followed as a natural consequence of the subsequent amelioration of the climate. While there is no sufficient evidence for such an extreme view, there is good reason to believe that the glaciation of these islands did result in diminishing and in altering the general facies of the pre-glacial flora. I need not review that succession of Pliocene and Pleistocene fossil floras which points to this conclusion: references are given in my first paper. It may be mentioned, however, that since the time of Forbes, who laid the foundations of the study of plant-geography in Britain in his classic memoir (5), both plant and animal geographers have connected the arctic element in our flora and fauna with the oncoming of those nival conditions which culminated in the Pleistocene Ice Age. But of the five sub-floras which, according to Forbes, comprise the total flora of Great Britain and Ireland, the arctic one (or Scandinavian type) is the fourth in terms of geological chronology. It was preceded by other three floras. Firstly, the Iberian type, which, although the most southern in character, is held to be the oldest, its extreme isolation in west Ireland being regarded as evidence of its antiquity. Secondly, the Armorican type, occupying south-east Ireland and south-west England, is related to the flora of the Channel Isles and

north-west France. Thirdly, the Kentish type, occurring in south-east England, is connected with the flora of north-east France. Only the fifth sub-flora, the general Germanic type, widely developed in the British Isles, is regarded as of post-glacial date. The continental connexions pointed out by Fôrbes are undoubtedly significant, but I am not in agreement with him when he assigns a pre-glacial origin to his first three sub-floras. The Pyrenean plants of west Ireland are not isolated units. They connect with other southern types both in Ireland and in the south and south-west of England. All of them form but the outlying and insular fringe of a flora whose home at the present time is west and south Europe. That such a southern flora survived in the land which is now Britain, when an ice sheet extended its margins close to the Thames valley and when the English Channel carried floating ice, seems to me more than improbable. That a hardy flora of boreal species, such as occupies the fringe of glaciated lands at the present time, did survive on the unglaciated areas of south Britain throughout the period of maximum cold is, however, extremely likely. The present flora of Greenland includes 416 vascular plants, a few of which occur as far south as the eastern United States, about the same latitude as the Mediterranean. But, so far as I am aware, no one has suggested that the southern species in the flora of Greenland survived the Pleistocene glaciation of that continent. Indeed, the whole flora is usually regarded as post-glacial. A recent and very exhaustive study by Fernald (4) has led to the conclusion, however, that certain species persisted in unglaciated parts of Boreal America throughout the Pleistocene. The argument is based upon the ascertained facts of geographical distribution and upon the occurrence of a striking endemism of the flora of the Gaspé Peninsula in the St. Lawrence which did not come under the influence of maximum ice-sheet development. South Britain may also have harboured a considerable flora throughout the glacial epoch, but that flora, I believe, could only have been a boreal one.

Excluding the arctic and boreal element, then, I picture the British flora as the resultant of numerous invasions from the mainland, coming from different directions. At least five invasions can be distinguished: (i) East Anglian, (ii) Kentish, (iii) Channel, (iv) Peninsular, (v) Connacian. It has been shown, in broad outline, that the areas within the British Islands which these names denote are connected with corresponding areas on the Continent. Into the details of the continental distribution, however, I have not entered, but it would be interesting to know how far the details correspond on both sides of the English Channel and the North Sea. In this connexion some observations have been published by Stomps (24), who gives data relating to the continental range of a number of English species which prove to be chiefly East Anglian. Arguments are advanced in favour of an invasion from the south-east to account for the arrival of these species in England. The valley of the ancient Rhine at the close of the glacial epoch is discussed

as a path of plant dispersal, and the range of a number of species on the Continent and in England is regarded as depending upon it. Moreover, the introduction into England of the river-bed species, with which Stomps is chiefly concerned, is referred to a date as recent as the oak period.

Again, as far as the evidence goes, the restricted floras of the south of England appear to be recent. Their present geographical range seems inconsistent with the idea that their arrival dates back to pre-glacial times. Nor is there fossil evidence in favour of this view. Clement Reid writes as follows: 'A hardy fauna and flora seem to characterise the period of the submerged forests; but the absence or great scarcity of characteristic survivors from a former period suggests that even the lowest of these deposits is far removed from the glacial epoch. The arctic species had already had time to die out, or had been crowded out; but the time had not been sufficiently long for the incoming of the southern forms which now characterise our southern counties.' The time factor which is here introduced is one to be borne in mind, and reference will be made to it later. A comparison of the present flora of south England and north France adds weight to the argument advanced by Clement Reid. The latter area possesses a large number of species not found in the former, although a few of them have reached the Channel Isles. Chevalier (3) contends that the differences cannot be explained on climatic grounds, and comes to the conclusion that the northward migration of species, after the glacial period was over, was interrupted by the opening of the English Channel.

If we regard the Kentish, Channel, and Peninsular invasions as post-glacial, it seems consistent to allow a post-glacial date for the immigration of the southern Irish flora, since there is evidence of a close phyto-geographical relationship between the south of Ireland and the Peninsula province in England. But the Peninsular flora is to a considerable extent Atlantic and Mediterranean, so that the migration which has furnished Cornwall and Ireland with certain peculiar floristic features has only to be shifted a few degrees west to provide the Connacian invasion of west Ireland from south-west Europe.

This picture of an advancing flora should be envisaged as a whole. Although built of several parts, I do not think these parts can be completely dissociated. One migration merges into the other, and plants from the south have met with others from the east. This tends to obscure, as we have seen, the working of the 'Age and Area' principle, and in the analysis of any flora along these lines it will always be helpful to trace, if possible, the invasion routes. Within the British Isles, each invasion appears as an integral part of a connected whole, indicating phases of a progressive movement. The succession seems to have been from west to east. The invasion which reached west Ireland is the oldest; that which reached East Anglia is more recent. But in my opinion they both belong to Quaternary times, the one

being the beginning, the other the end of a long-continued process which has been going on for thousands of years.

The migratory movement, thus briefly sketched, raises the question of the former existence of land connexions during the Quaternary. Even if we allow for the occasional dispersal of plants over the sea and their establishment in insular areas which show a geographical connexion with the nearest mainland, we are confronted with the difficulties presented by animals. Both the Irish and English faunas include a number of species which, like some of the plants of west Ireland, have their nearest continental homes in south-west Europe. For numerous zoogeographical details reference may be made to Scharff's 'European Animals' (20). I shall quote but one recent record which relates to the discovery of a distinct species of a white-toothed shrew of the genus *Crocidura* from the Scilly Isles. The account is given by Hinton (8),<sup>1</sup> who states that the genus has a wide distribution throughout Africa and the warmer parts of Europe, extending westwards to the Atlantic coast of Europe and the Channel Isles. Although the specimen from Scilly is not identical with any species hitherto described, its occurrence in these islands represents a noteworthy extension of the genus which must have happened when land connexions were in existence. Facts such as these have to be taken into account by the botanist, for they suggest the former existence of land to the west of the present coast-line of Europe. Archaeological research indicates a connexion between England and the Continent down to comparatively recent times. Moreover, Reid Moir (12) states that Man flourished in the past in England, as elsewhere, during warm interglacial epochs, and to regard all his remains found in this country as referable to post-glacial times is erroneous. Man existed prior to, and survived the rigours of, the Ice Age.

I have already stated that south England, which was ice-free, carried in all probability an arctic vegetation even during maximum glaciation. It is, therefore, relevant to inquire when maximum ice-development occurred in Britain in order to have some idea of the time which has elapsed since the margin of the ice-sheet lay along the Thames Valley. This seems to have coincided with the Mindelian glaciation of the Alps, towards the close of which a high northern and arctic marine fauna appeared in the Mediterranean, where the stage is known as the Sicilian, and which has been said by Professor Sollas (21) to be the only sign of an Ice Age. Subsequently, according to Brooks (2), 'Europe must have passed through a series of stages of amelioration, of which traces can be found here and there, though the details are lost to us. Ultimately, temperate conditions again prevailed, and for a very long time, *approaching a quarter of a million years*,<sup>2</sup> Europe

<sup>1</sup> I am indebted to Dr. Waterston, of the British Museum, for drawing my attention to this paper.

<sup>2</sup> Italics mine.

cannot have differed greatly from present climatic conditions.' In fact the climate was probably warmer than at present. A readvance of the northern ice-sheet brought this long warm interval to a close, and the climate became colder again, but the point of greatest moment is the date of maximum glaciation, and altogether it has been estimated that a period of approximately 400,000 years have elapsed since the ice-development attained its Mindelian climax.

This is not an inconsiderable period in the history of any flora and, in the case of our own I believe it to be sufficiently long to allow of those numerous changes which have led finally to the emergence of our flora at its present state of distribution. We cannot follow the details, for these are buried in the past. But, making use of the maps which have been constructed, we may trace, as the result of an ameliorating climate which set in first in the south-west of Europe, a northward movement of plants along a coastal belt whereby they reached ultimately those areas they now occupy in south and west Ireland. This represents an early phase of the immigration of the southern members of our flora. As the Irish and English Channels opened, the migratory path would become more circuitous. The approach to Ireland was first cut off, but for plants still reaching England it made possible that northward advance of a number of species along the west of Britain, some gaining the north-west of Scotland. Later, the opening of the Straits of Dover allowed a number of these southern types to creep along the northern shores of Europe as far as Norway. But meanwhile the main centre of dispersal from Europe was shifting eastwards and the bulk of the Germanic flora, which forms so large a part of the flora of Britain, continued to reach England from the south-east. This continued as the chief invasion so long as there was a land connexion with the Continent. The separation occurred about 6,000 years ago. Since then many plants have established themselves in Britain, but if the history of these relatively recent arrivals could be unfolded, it would be found that man has played a large part in widening their range, if not actually responsible for their introduction. Yet they are added to the list of so-called British plants. If the aliens, denizens, and colonists were removed, our native flora would not be very large in number of species.

If the general outlines of dispersal suggested in the foregoing paragraphs approximate to the actual sequence of events, it will be realized that disintegration of a former westward extension of the European main may have had a profound effect in modifying the range of species. It will be one cause of discontinuity in the distribution of plants, and to this I attribute the localization of the so-called Lusitanian species in Ireland. The wider distribution in Ireland than in England of a southern type like *Euphorbia hiberna* may not be without significance. It ranges in south and west Ireland from Waterford to Donegal, though not in County Clare, while in



England it is confined to three Peninsular vice-counties. The position of the Atlantic type, *Simethis bicolor*, is more critical, for it is restricted to eight or nine miles of the coast of south Kerry along the Kenmare river and to a single station near Bournemouth in Dorset. Should it disappear from its English locality first, which seems likely, there will be added, as Stapf points out, another species to the peculiar Irish element of the British flora. The surprising thing, as Goodchild (6) remarks, is that this element is not larger than it is. Already it would appear that *Diotis maritima*, a southern littoral species, which was known from several counties in England from Suffolk to Anglesey, has become extinct and is now confined to the south-east shores of Ireland (Waterford and Wexford), where it is very rare.

While a few species are restricted to Ireland, the majority of the southern Irish plants seem to have advanced through south-west England. A migration from the Continent following this direction would include the Channel Islands, and evidence of the continuance of this invasion after the English Channel had opened would be the occurrence in these islands of southern species which have not reached Britain. The flora of the Channel Isles includes twenty-seven flowering plants not recorded from Britain. Marquand (9) gives a list of seventeen from Guernsey and remarks that they are by no means common on the adjacent French coast. On the contrary, not one occurs there plentifully, and five or six do not occur in Normandy at all. In fact the main centre of these Channel Isles species is south-west France, although several extend far eastward along both north and south shores of the Mediterranean. Several occur also in the Canaries, and a few reach Belgium and Holland, but only five penetrate into central Europe. These facts suggest that these Sarnian species belong to that same migration from the south which provided England with a small assemblage of plants concentrated in the south-west counties. There is further evidence in support of this view. If the proportion of Sarnian species in the rare provincial floras of England be determined, we find that the figure is highest for the Peninsula province, and higher for the Channel than for the Thames. In other words, the restricted Peninsular flora is the one which is most Sarnian in character.

Having regard to the available data, much of which has been presented cartographically, I have been drawn to the conclusion that the non-boreal flora, which migrated into these islands after the retreat of the ice, began by establishing itself in the south-west and gradually progressed eastwards. Disintegration of area has led to discontinuity in the range of a few species, and many members of the southern element seem to be restricted to south and west Britain by climatic factors, while others, such as littoral species, are restricted by the nature of their habitat. But quite apart from these limitations, the earlier stages of the invasion are largely overshadowed by the succeeding stages, which were of longer duration. The prolonged immigra-

tion from the south-east of a central European flora, more aggressive in character and less restricted by climatic conditions, has dominated the other migrations and has impressed itself as the chief invasion in the establishment of the flora of Britain.

#### SUMMARY.

This paper is a third contribution to a study of the geographical distribution of certain restricted portions of the British flora. It deals, firstly, with those plants that are found only in Ireland, and, secondly, with those which occur in England, Wales, and Ireland, but not in Scotland.

Attention is drawn to the fact that the Hibernian plants, which form a small but puzzling group of sixteen species, are with few exceptions outlying members of a south European stock, which, taken as a whole, forms a well-defined element in the flora of Britain, an element that has been fully described by Dr. Stapf. A map shows the distribution of the Irish members, and while they are absent from England, they connect with their main continental area by a series of progressive steps.

The 'Anglo-Irish' group is a larger one, numbering sixty-eight species. Their mass distribution indicates a general prevalence in France and south Europe, while in England the south and south-east counties show the greatest density. The main outlines of distribution are not unlike those seen in the case of entirely 'English' species previously dealt with, but in the 'Anglo-Irish' group a western tendency expresses itself. Within Ireland the assemblage is distributed rather unevenly.

An analysis of the rarity of the group is then given. The range of the rarer species points to a close connexion between south-east Ireland and south-west England. This is borne out especially by those Munonian species whose head-quarters are in south Ireland. Reference is made to other 'types of distribution' in Ireland, and a few species of wide range outside Ireland are peculiar in being confined to the west of the island.

The paper concludes with a general discussion in which the view is expressed that the immigration from the Continent of the non-boreal flora of Britain may be traced in the several distribution maps which have been presented in this and earlier papers. Five invasions are defined which have shared in the building of our flora over a long period subsequent to the time of maximum glaciation. The invasions reaching the south-west of England and Ireland are regarded as the beginning of the re-immigration process, but they merge into, and are inseparable from, the later stages when the chief centre of dispersal had moved eastwards on the Continent. A prolonged invasion from the south-east became the dominant one and accounts for the preponderance of the central European element in our flora.

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# On the Occurrence of Parichnos in Certain Conifers.<sup>1</sup>

BY

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AND

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With Plate XXXIII and seven Figures in the Text.

THE group containing those ancient arboreal Lycopods known as Lepidodendroids and Sigillarians has been known since 1891 to possess aerating canals, or at any rate strands of lax tissue, accompanying the foliar traces from the cylinder outwards and sometimes, if not always, bifurcating at the base of the leaf. These strands or canals apparently became continuous with the spongy parenchyma of the mesophyll of the leaf. They were called by their discoverer, Bertrand,<sup>2</sup> parichnos. Much literature has appeared in regard to their structure and occurrence, and the question of their function is still more or less an open one so far as the fossil forms are concerned.

These structures were observed later by Potonié to be related to paired lenticel-like organs, which became more and more marked with the increased age of the stem which bore them. We shall return to these at a later stage. The occurrence of parichnos-like structures has been described in the case of other lycopodineous forms, namely, *Isoëtes* and *Lycopodium*.<sup>3</sup> It is the intention of the present article to call attention to the distribution of parichnos outside the Lycopodiales, living and extinct.

Pl. XXXIII, Fig. 1, shows the external appearance of the stem of a fir

<sup>1</sup> Contribution from the Laboratories of Plant Morphology, Harvard University.

<sup>2</sup> Bertrand, C. E.: Remarques sur le *Lepidodendron Harcourtii* de Witham. *Mém. des Facultés de Lille*, ii, 1-159, 1891.

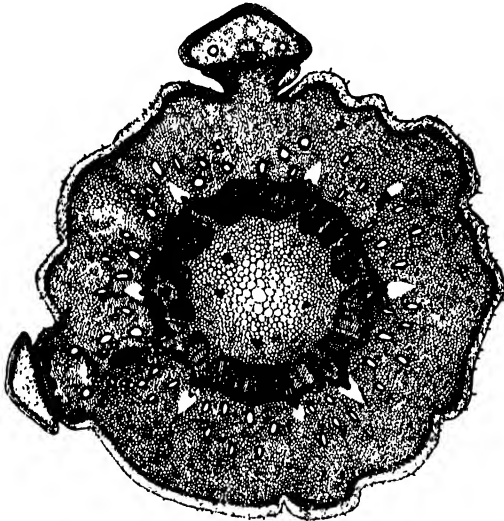
Coward, Katherine H.: On the Structure of *Syringodendron*, the bark of *Sigillaria*. *Mem. Man. Litt. and Phil. Soc.*, li, No. 7, 1-6, 1906-7.

<sup>3</sup> Scott, D. H.: *Studies in Fossil Botany*. London, 1st edition, 1900.

Jones, C. E.: The Morphology and Anatomy of the Stem of the Genus *Lycopodium*. *Trans. Linn. Soc., Lond., Bot. II*, vii, 15-35, 1905.

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from which the leaves have fallen. In the smaller twigs in the middle the leaves are only recently deciduous, whilst in the larger branch on the right the foliar structures have fallen for some time. It will be noted in the case of the older branch that the leaf-scars have apparently increased in size. This is due to the formation of lenticular tissue in the region of the scar. In old stems of the Balsam Fir and other firs, these lenticular organs, related to the original leaf-scars, often develop to great length in the transverse direction, and are very conspicuous objects on old trees in which rhytidome formation has not yet begun. The correlation between the lenticels and the leaves



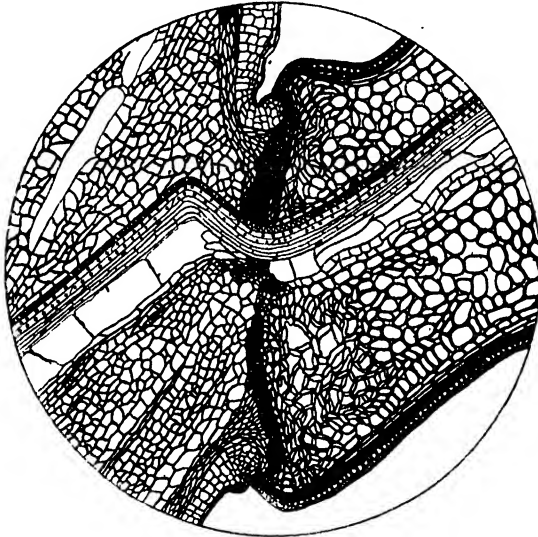
TEXT-FIG. 1. For description see text.

in the fir is of considerable interest from a number of standpoints. In the first place, it is significant that the leaf, which is practically the sole aerating organ in those abietineous Conifers which are without decurrent leaf bases, is replaced at its fall by an aerating structure, the lenticel. It is highly significant in this respect that the epidermis of the stem in the fir and in the pine, as well as other Abietineae, is devoid of the stomata which are so commonly present on the stems of the Angiosperms. Secondly, the aerating function of the lenticel is obvious in this case and beyond question. The lenticel is moreover related, as we shall show later, to aerating canals within the cortex of the stem, which correspond in general topography and organization exactly with those in the *Lepidodendroids*.

Pl. XXXIII, Fig. 2, shows the surface of the root in the Balsam Fir. It will be noted that lenticels are present as in the stem. Out of one of them a root can be seen emerging. Sections through smaller lenticels on smaller or larger roots show an intimate relation between the appendage and the

lenticel. It is of course well known that stomata are absent on roots and that their place is taken by lenticels. Commonly in the fir, the pine, and other related Conifers, the lenticels appear only on the surfaces opposite the protoxylem points of the primary wood. It is obvious in this figure and the preceding one that there is an intimate relation between lenticels and appendages.

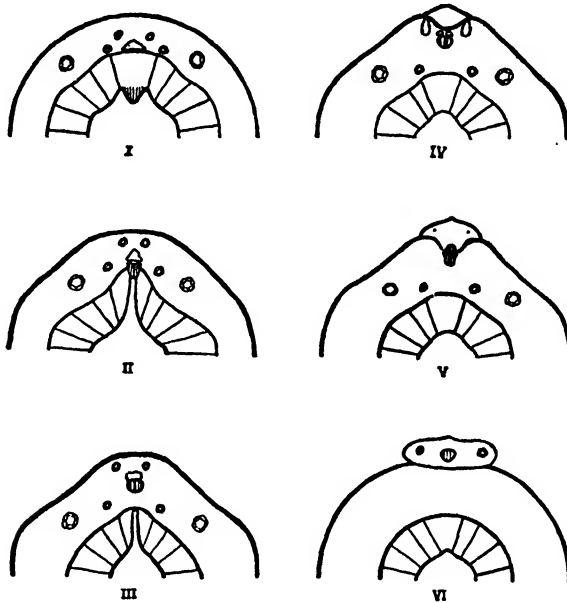
It will be convenient at this stage to refer to the internal relations of the lenticels and appendages in the fir. Text-fig. 1 reproduces, slightly diagrammatically, a transverse section of the main axis of a seedling of the



TEXT-FIG. 2. For description see text.

Balsam Fir. The woody cylinder is marked by indentations along the margin of the pith, which correspond to outgoing leaf-traces. It will be observed that these departing traces are accompanied both in their course in the cylinder and outwardly in the cortex by an air-canal, which appears white in the illustration. Similar conditions can be seen less distinctly in Pl. XXXIII, Fig. 3, representing a photomicrograph of a transverse section of a branch of the Balsam Fir. Pl. XXXIII, Fig. 4, shows a portion of Fig. 3 more highly magnified. The illustration reproduces the departure of a leaf-trace from the surface of the woody cylinder. Outside the leaf-trace lies a clearly defined air-canal or parichnos. Pl. XXXIII, Fig. 5, shows a leaf-trace which has passed some distance into the cortex, but which is still accompanied by the aerating canal or parichnos. Pl. XXXIII, Fig. 6, shows a vertical section cut radially through the length of a leaf-trace. It will be observed that the outer lower surface of the leaf-trace is accompanied by an air-canal, in which lie certain shred-like remains of broken-down cells. It is quite

clear, from the comparison of older and younger stems, that the parichnos in firs and allied forms arises lysigenously. It can be seen that the parichnos, as such, ends at the base of the leaf in the region of what will be later the absciss periderm. In the leaf base the position of the parichnos in the stem is occupied by a mass of transfusion tissue lying on the lower, outer side of the fibrovascular bundle. The parichnos is not related to this transfusion tissue, but to the spongy mesophyll occupying the lower surface of the leaf. Text-fig. 2 shows a somewhat diagrammatic drawing in which



TEXT-FIG. 3. For description see text.

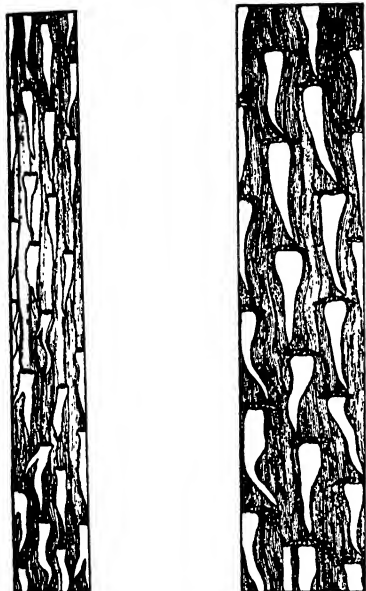
the topographical relations of the parichnos appear more clearly. As pointed out above, it ends at the base of the leaf and becomes lost in the loose mesophyll of the foliar organ. The photograph described above, as well as the text-figure, were made from a young stem cut in July. In spite of this, however, the periderm, which will later interrupt the relations between the leaf and the stem, has already made partial progress in development.

Text-fig. 3 shows diagrammatically the topography of the parichnos as it passes outwards and upwards from the surface of the cylinder to accompany the leaf-trace in its course through the cortex. Drawing I represents the parichnos in contact with the surface of the cylinder. In II the leaf-trace has begun to free itself and to pass outward accompanied by the parichnos. In III still further progress has been made, and the outline of the parichnos shows a median indentation on its outer surface. In IV the parichnos is

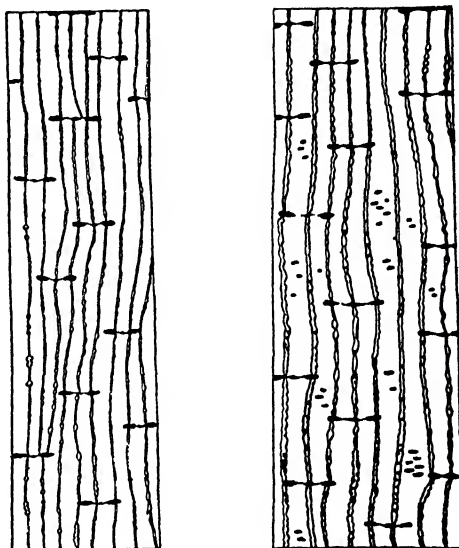


divided into two, a condition which parallels in an interesting way the situation found frequently in extinct arboreal Lycopods. In V the divided parichnos is practically obsolete, and in VI the base of the leaf, now almost free from the stem, shows no evidence whatever of the air-canal or parichnos.

We may now pass to the consideration of the pines, which constitute with a high degree of probability the oldest living representatives of the Abietineae. Text-fig. 4 represents branches of the Pitch Pine, *Pinus rigida*, from which the short shoots and their subtending bracts have fallen. It



TEXT-FIG. 4. For description see text.



TEXT-FIG. 5. For description see text.

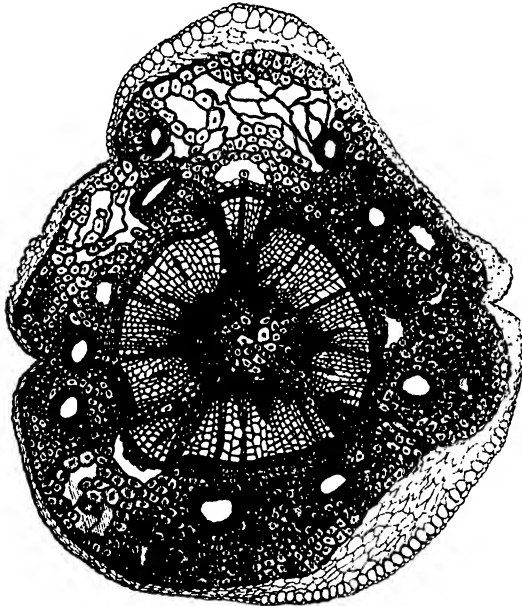
will be observed that, particularly in the larger and older branch, two wing-like lenticular structures appear, one on either side of the fallen short shoot and its subtending branch. The situation in the soft pines, such as *Pinus Strobus* and its allies, is even clearer. Text-fig. 5 shows the surface of the stem of White Pines which are several years old and in which the short shoots are consequently deciduous. In the smaller branch on the left the scars of the fallen appendages are subtended on either side by a lenticel, which is represented as a black oval in the diagram. The longitudinal lines which meander from top to bottom in the figure represent the position of the resin canals, which cause conspicuous elevations on the surface of the stem. In the item on the right of this figure is shown an older stem where the lenticels related to the appendages are supplemented by others, represented by dark dots, having no such relations. Pl. XXXIII, Fig. 7, shows a photograph of the superficial tissues of *Pinus Strobus*, which have been

removed by maceration in such a way as to show the topographical relation of the scars of the appendages and the lenticels. In the centre above is the scar of the short shoot, and below it lies the cicatrix of its subtending bract. On either hand can be seen very distinctly and clearly circumscribed lenticels. It is of interest to compare this figure with Pl. XXXIII, Fig. 9, representing the paired lenticel-like organs in *Syringodendron* as figured by Potonié. The resemblance in this feature, between the two forms which are so remote in relationship, is very striking. It will be noted on examination of Pl. XXXIII, Fig. 7, that no stomata are present. Pl. XXXIII, Fig. 10, shows a photograph of the macerated epidermis of the stem of a *Lepidodendroid* from the Russian 'leaf-coals'. Neither the epidermis of the stem in the *Lepidodendroids* nor that of *Pinus* bears any stomata, and as a consequence aeration is carried on exclusively by the leaves while these are still present, and subsequently, after their fall, by lenticels related intimately to their bases.

We may now turn our attention to the internal organization of the pine in regard to the presence of parichnos. Pl. XXXIII, Fig. 8, shows a photograph through the cylinder of a pine seedling, in which the primary leaves are still present. It will be noted that four leaf-traces are in various stages of departure from the surface of the cylinder. In each instance the trace is more or less clearly subtended by a cavity in the cortex, the parichnos. On the right of the figure is shown a leaf-gap, which corresponds to a trace which has already passed out. The parichnos in the case of the pine has a much abbreviated course, and does not extend so far towards the base of the leaf as is the case of certain firs. Text-fig. 6 shows the general topography of a young axis of the White Pine. The relation between air-canals or parichnos and leaf-traces stands out more clearly than in the photograph. The parichnos in *Pinus* is not confined to the soft pines, but occurs also in the hard pines. In the latter group it is in general less recognizable on account of the fact that there are numerous cavities in the cortex other than those representing the parichnos. In the White Pine the structures in question stand out with particular clearness on account of the absence of considerable cortical intercellular spaces. Moreover, the parichnos is not a structure confined to the young stage of the development of the pine, but is present also in older parts of the tree and when the short shoots are well established. It is not always, however, as distinct in branches of the adult as it is in the axis of the seedling, where it is intimately related to the course of the primary leaves.

It naturally occurred to us to examine superficially and anatomically the stems of other representatives of the Abietineae. Only in *Picea* and *Larix* were we able to observe internal evidence of the presence of parichnos. Pl. XXXIII, Fig. 11, is a photograph of a departing leaf-trace in the spruce, *Picea rubra*. It will be observed that there are certain large cells on the

outside of the leaf-trace. These tend to break down and produce more or less imperfect canals corresponding to those more clearly seen in the pine and the fir. Similar observations were made in the case of several other species of *Picea* as well as in *Larix*. In *Picea* and *Larix* the parichnos shows more distinctly in young and vigorous branches. One finds, on the younger axes of both *Picea* and *Larix*, lenticels corresponding in position to the fallen appendages. These are at first strictly related to appendages, but very soon others, not topographically in relation to the appendages, make their appearance. In other abietineous Conifers appen-



TEXT-FIG. 6. For description see text.

dicular lenticels are present on the stem, and are sooner or later supplemented by others which are not appendicular.

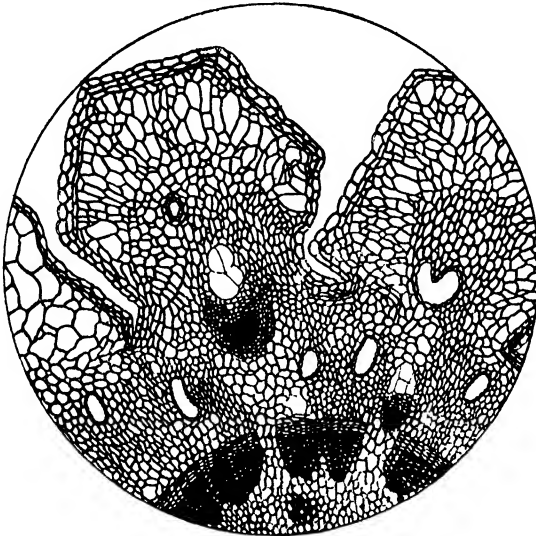
It is of interest in the present connexion to inquire whether parichnos is present in Conifers other than the Abietineae. We have examined branches of various ages in *Araucaria imbricata* and *Araucaria Bidwillii* without having detected any indication whatever of the presence of air-canals or parichnos accompanying the leaf-traces. Similar results were reached in the case of *Agathis australis*. The results for the araucarian Conifers are consequently negative. Possibly investigation of seedlings would prove more fruitful in this respect.

A number of Taxodineae were likewise studied, namely, *Sciadopitys*, *Sequoia*, and *Cunninghamia*. In no case could parichnos be detected. The same statement holds for the genus *Athrotaxis*.

In the Cupressineae, *Libocedrus*, *Chamaecyparis*, *Juniperus*, and *Thuja* were investigated with negative results.

The examination of several species of *Podocarpus* likewise gave results of a negative character. It is possible that in this and in other cases the study of seedlings will be more fruitful of results.

The reader will recall from earlier paragraphs that the Abietineae, in contrast to other Conifers, are characterized by the presence of air-canals passing outwards with the leaf-traces, and terminating either at the base of the leaf or earlier. These canals in their relations correspond exactly



TEXT-FIG. 7. *Larix*, showing a departing leaf-trace and its parichnos.

with similar canals described in fossil and existing members of the Lycopodiales. It is of interest to note that the subterranean stem of some of our existing species of *Equisetum* is characterized by the presence of lenticels, which are in communication with the aerating cavities in the internal cortex. The senior author has called attention to this situation in his memoir on the genus *Equisetum*.<sup>1</sup> Possibly a more careful investigation of fossil remains of the Equisetales, in a good state of preservation, would reveal the presence of parichnos or similar structures. It is not improbable that, if they are discovered later, they will be found in relation to lenticels precisely as has turned out to be the case in the arboreal Lycopods and in certain Abietineae. It seems likely that the parichnos corresponds to the needs of aeration of plants existing either under aquatic or amphibious

<sup>1</sup> Structure, Development, and Affinities of the Genus *Equisetum*. Mem. Bost. Soc. Nat. Hist., 1900.

conditions. It is a well-known fact that herbaceous water plants of the present epoch are characterized by the presence of numerous air-canals in the internal cortex. It is very generally agreed that the ancestors of our existing land plants came originally from forms adapted to wet or even submerged soil. In many instances these lower forms are known to us to have possessed air-spaces in their cortex. This is notably the case in the lepidodendroid forms and in the *Calamites*.

It has been pointed out by one of us that the surviving genus *Ginkgo* is connected by many remarkable features of resemblance with the Abietineae. These are as follows: The reproductive structures in *Pinus* and *Ginkgo* are strikingly alike. The sporangia occur in pairs on the appendages and on the morphologically lower surface. The opening mechanism of the microsporangia in the two groups consists of tracheid-like cells in intimate relation to the transfusion tissue of the fibrovascular bundle. The winged microspores of *Pinus* and other Abietineae have their counterpart in the winged pollen grains which we have observed to be present in *Ginkgo*. It is of interest in this connexion to point out that similarly winged microspores were present, not only in certain Cordaitales, but have been found likewise in the pollen chamber of certain fossil seeds of unsettled affinities, notably *Stephanospermum*. The woody structure of *Ginkgo* corresponds to that of *Pinus* and other related Conifers, and not to that of the Cordaitales or that aberrant group of Conifers known as Araucariineae. In both *Ginkgo* and *Pinus* are found those remarkable structures lying horizontally between the opposite pits known as bars of Sanio. These occur only in the secondary wood, although erroneous statements have been made as to their presence in the primary xylem. Bars of Sanio occur in the Gnetales, and, as has been recently pointed out in a contribution from this laboratory, may be seen in the secondary wood, particularly the root wood of certain lower Angiosperms. Another interesting feature of resemblance between *Ginkgo* and *Pinus* is the phenomenon of 'Rotholz'. This is a highly coloured mechanical tissue which in the past has been noted as a characteristic feature of the wood in the Conifers. It is not without significance that the same type of mechanical tissue is found in *Ginkgo*. It will appear from the many resemblances enumerated above that *Ginkgo* and *Pinus* are very closely related. This view is new, and runs counter to accepted opinions based on purely Palaeozoic evidence. On account of the numerous and significant resemblances between *Ginkgo* and the Abietineae, described above, it becomes a matter of interest to inquire as to the presence of parichnos in *Ginkgo*. The superficial conditions in *Ginkgo* do not warrant a favourable expectation in this respect, because the lenticels on the long shoots are late in appearing and have no definite relations to the appendages. A careful search, however, has been made by the junior author, with the result that

parichnos-like structures have been found to a limited extent in relation to the traces of the earliest leaves or bud-scales borne on the short shoots. They are less obvious, but still present, in relation to the traces of the leaves attached to the long shoots. The mass of leaves related to the short shoots, and all of those beyond the first few which are formed, are entirely without parichnos. This is not surprising, however, since parichnos does not manifest themselves in relation to the leaves of the short shoots in *Pinus*. It would have been of interest to investigate conditions in the seedlings of *Ginkgo*, but none of these at the present time are at our disposal. Pl. XXXIII, Fig. 12, is a photomicrograph of one of the lower leaf-traces of the short shoot in the case of *Ginkgo biloba*. It will be observed that there is a definite cavity or air-space along the outer and lower surface of the trace, and this we consider to correspond with the similar air-canals found in certain Abietineae and in certain Lycopodiales. It thus appears that *Ginkgo*, corresponding in so many other features with *Pinus*, presents likewise a resemblance in its possession of air-canals or parichnos.

The general development of the parichnos in Abietineae and in *Ginkgo* indicates a structure which is obsolescent. In *Pinus* the parichnos shows its best development in the seedling, and in other Abietineae it can be more clearly made out in the young conditions than in the old. We have noted above that in the case of *Ginkgo* the parichnos is confined to the traces of the lowermost leaves of the short shoots, and to the leaves of the probably more primitive long shoots. It seems thus obvious that the parichnos is a structure derived from the past which has almost or quite disappeared in the Gymnosperms. The question arises in this connexion as to the significance of the parichnos as an indication of phylogenetic affinities. A parallel case is apparently presented by the primary wood. The development of the primary wood in more ancient forms and in the roots of all woody plants is centripetal. In contrast to this condition, we find in the stems of modern forms, and for the most part in the leaves of modern forms, a centrifugal development of the wood. The presence of centripetal wood indicates antiquity, but not necessarily close relationship. For example, in a fossil form, *Prepinus*, described by one of us, the leaves have true centripetal wood, a feature more archaic than is found in the leaves of any other known Conifers, living or extinct. The Lycopods in general are characterized by the presence of centripetal wood. It would, however, be ill-advised to consider that the presence of centripetal wood in Abietineae and Lycopodineae is necessarily an indication of relationship between the two. All that can be said concerning the centripetal wood is that it is undoubtedly an ancient type of wood. The presence, however, of centripetal wood does not, in the absence of other evidence, justify a conclusion of close affinity.

The parichnos of the Lepidodendroids and the Abietineae seems to

belong in a similar category. If we attempt to imagine the mode of origin of the parichnos, we have the clearest evidence in the case of our modern herbaceous water plants, which are characterized by the presence of air-canals in the inner cortex. There is no reason whatever to believe that our living aquatic Angiosperms are primitive Angiosperms. Their general anatomy indicates, on the contrary, that they have come from terrestrial forms. In spite of that fact, however, they show clearly the influence of an aquatic habit on internal organization, and thus throw light on the mode of origin of the anatomical structures characteristic of water plants. They are thus useful, paradoxical as it may seem at first, in giving us some conception of the condition of organization of the early vascular plants, which were just emerging from the fresh waters to settle themselves on land. Doubtless many of the characteristics of our more primitive land plants can be traced to this previous amphibious existence.

To our minds, the parichnos has its explanation in the amphibious mode of life of the earlier vascular plants. It is a well-known fact that the cortex of existing water plants is cavernous with air-spaces, which arise either lysigenously or schizogenously. To our way of thinking, these are the counterparts of the parichnos. In other words, the parichnos represents a primitive method of aeration which has persisted in the Lycopods, in certain Conifers, and in *Ginkgo*. It is a generally accepted position that conservative forms perpetuate ancestral conditions. One of us has brought forward abundant evidence for the primitiveness of the genus *Pinus*, which is certainly at the present time the most ancient tree of our northern forests. Its only possible rivals are the Kauris and Araucarias, which are now banished to the Southern Hemisphere. *Ginkgo* and *Pinus*, although long living on land, appear to have retained the ancient mode of aeration by means of the parichnos. The parichnos became related later to lenticels in juxtaposition to the appendages.

The presence of parichnos, when accompanied by other valid features of resemblance, furnishes a strong clue as to relationship. We do not consider the common possession of parichnos by certain lower Conifers and Lepidodendroids to indicate anything more than the fact that the Conifers are an ancient group. There seems to be no convincing general evidence that their ancestors were in close relation to those of the Lepidodendroids. In the case of the Abietineae and *Ginkgo*, however, the situation was very different, because, as we have noted above, there are a host of striking and highly significant features of resemblance between *Ginkgo* and *Pinus*. The occurrence of parichnos vouches for the antiquity of the Abietineae and *Ginkgo*, and the other features of resemblance, which are more categorical and convincing, seem clearly to indicate that they had a common origin.

## SUMMARY.

1. Aerating canals corresponding to those found in certain lepidodendroid forms are here described as occurring in *Pinus* and other Abietineae.

2. These canals, as in the Lepidodendroids, are related to the leaf-traces and accompany them on their lower side through the cortex.

3. It has been observed in recent years that there was a relation between the parichnos and lenticels in the Sigillarian and allied types.

4. We have demonstrated a similar relationship between paired lenticels or virtually paired lenticels and the parichnos in certain Abietineae.

5. The presence of parichnos and lenticels appears to be correlated definitely with the absence of stomata in the epidermis of the stem. This is notably the case with *Pinus* on the one hand and *Lepidodendron* on the other.

6. The parichnos constituted a primitive organ of aeration.

7. The presence of parichnos indicates antiquity and conservatism, but not necessarily relationship.

8. We have noted the existence of parichnos in *Ginkgo* as well as in the Abietineae and the Lepidodendraceae.

9. On account of numerous other features of resemblance between the Abietineae and the *Ginkgo*, we regard *Ginkgo* and *Pinus* as having had a common origin in the past.

10. We do not consider on the evidence at present available that there is any close relationship between any Conifers and the Lepidodendraceae.

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HARVARD UNIVERSITY.

## EXPLANATION OF PLATE XXXIII.

Illustrating Professor Jeffrey's and Professor Wetmore's paper on the Occurrence of Parichnos in certain Conifers.

Fig. 1. Surface of axes of different age in *Abies balsamea*. To the left is a leafy branch; in the centre one which has just lost its leaves; to the right an older trunk. (Photograph.)

Fig. 2. Surface of the root of *Abies balsamea* facing one of the two protoxylem groups. Lenticels may be seen, and out of one of these a root is growing. (Photograph.)

Fig. 3. Transverse section of a young yearling shoot of *Abies balsamea*, slightly magnified. (Photograph.)



Fig. 4. Transverse section showing the exit of a leaf-trace from the cylinder in the Balsam Fir and its related parichnos. (Photograph.)

Fig. 5. A leaf-trace in the Balsam Fir towards the outer margin of the cortex showing a parichnos on its outer side. (Photograph.)

Fig. 6. A vertical section through a leaf-trace showing the parichnos on the lower outer surface of the trace. (Photograph.)

Fig. 7. Surface view of two lenticels, and between them the scars of short shoot and bract in *Pinus Strobus*. (Photograph.)

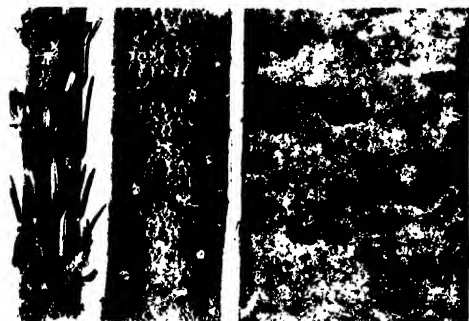
Fig. 8. Transverse section through the seedling stem of the White Pine, *Pinus Strobus*, showing the presence of parichnos in relation to the leaf-trace. (Photograph.)

Fig. 9. Copy of a figure showing lenticels in relation to the leaf-traces and parichnos in *Syringodendron*, after Potonié. (Photograph.)

Fig. 10. Photograph of a macerated epidermis of *Ulodendron* showing the leaf bases represented by holes. (Photograph.)

Fig. 11. Photograph of the young stem of *Picea rubra* showing a parichnos in relation to a leaf-trace. (Photograph.)

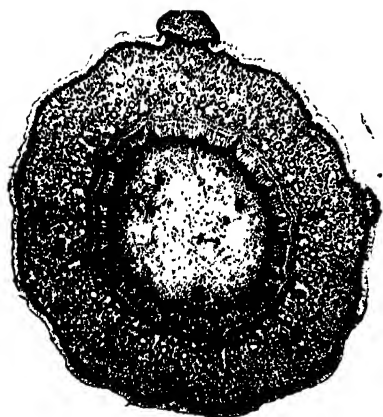
Fig. 12. Photograph of the trace of one of the basal leaves of the short shoot in *Ginkgo biloba*, showing a parichnos on the upper surface. (Photograph.)



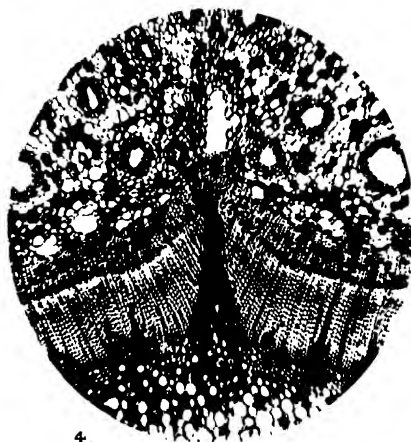
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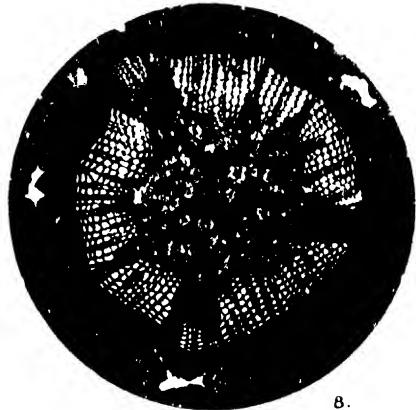
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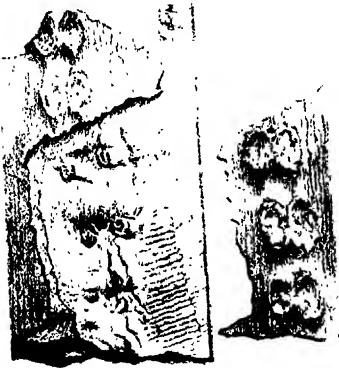
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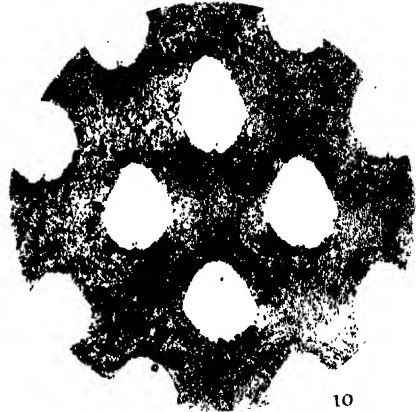
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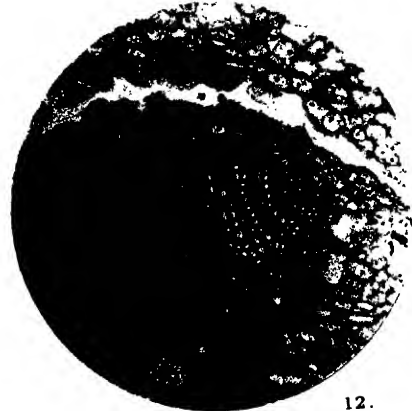
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Huth col'



# The Uredo Stage of the Pucciniastreae.

BY

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With Plate XXXIV and twenty-one Figures in the Text.

## INTRODUCTION.

AMONG the more interesting rusts (Uredinales) are those that occur alternately on Ferns and Conifers. Association with hosts of ancient lineage is generally considered to be good *a priori* evidence of antiquity and primitiveness. Granted that these rusts possess characters which are primitive, there is thus provided an excellent working hypothesis for the solution of two important interrelated problems presented by the Uredinales, namely, the origin of heteroecism and that of natural affinities. This method of attack has been employed in a recent paper (Arthur, 2) on the phylogeny of the rusts. Arthur supposes that the more primitive rusts are likely to be found upon the more ancient hosts; and, since the Ferns are undoubtedly the most ancient hosts of rusts, a study of fern rusts should give some clue to the character of primitive forms.

The most familiar rusts of ferns belong to the Pucciniastreae, a sub-family of the Melampsoraceae characterized by indehiscent teleutosori and aecidia of the peridermium type. Concerning the uredosori of the Pucciniastreae there exists considerable difference of opinion. Included also in this sub-family are certain rusts of flowering plants. However, as far as life-histories are known, the alternate stages of all the rusts of this group—those of Filicales and Angiosperms alike—occur on Conifers. Host relationships of this kind among rusts morphologically similar are surely of considerable phylogenetic significance.

Naturally, investigators have searched for criteria by which the fern rusts of the Pucciniastreae might be differentiated from those on flowering plants. On the basis of supposed differences in the uredosori, various steps

have been taken towards a natural grouping of this kind. For example, Magnus (36) transferred the fern rusts of the genus *Melampsorella* to a new genus, *Milesina*, in the belief that their uredospores are pedicellate while those of the angiospermous rusts are catenulate. Again, Ludwig and Rees (29) proposed a natural division of the Pucciniastreae into two groups, those on ferns (including the genera *Uredinopsis*, *Hyalopsora*, and *Milesina*) and those on flowering plants (*Melampsorella*, *Pucciniastrum*, *Thecopsora*, *Melampsoridium*, and *Calyptospora*). The rusts in the former subdivision were believed to have pedicellate uredospores. Concerning the latter group, Liro and Magnus had previously reported catenulate uredospores for *Melampsorella*; Ludwig and Rees found what they interpreted as catenulate spores in the uredosorus of a species of *Pucciniastrum*, and were thus led to make their suggestion of a natural grouping. On the other hand, Bell (4) has described what he regards as catenulate uredospores for *Uredinopsis*—a genus of fern rusts.

These references suffice to indicate that the literature dealing with the uredosorus of the Pucciniastreae is in an unsatisfactory condition. In addition to the question of the origin of uredospores, there are other matters which await elucidation: for example, the nature and origin of the uredoperidium and the occurrence of pores in the uredospores. Until these and various other points are satisfactorily settled, little can be concluded about the natural affinities of the Pucciniastreae or concerning primitive rusts.

This paper is concerned primarily with the uredosori of various members of the Pucciniastreae. In addition, a rather careful histological study of haustoria has been made. Fourteen species, representatives of six genera of the Pucciniastreae, have been investigated. These species are listed in the accompanying table, together with hosts and dates of collection. Three additional genera belong to this group of rusts, namely *Calyptospora*, *Necium*, and *Melampsoridium*. Of these, the first two do not form uredosori and consequently do not come within the scope of the main subject of this paper. Material of *Melampsoridium* suitable for histological study was not available.

#### MATERIALS AND METHODS.

The materials for this investigation were obtained mainly from the Timagami Forest Reserve, Ontario. For these the writer is greatly indebted to Professor J. H. Faull, Mr. G. D. Darker, and Mr. W. R. Watson, who exercised foresight and vigilance in locating representative forms, and who employed great care in selecting and fixing suitable specimens. One species, *Hyalopsora Polypodii*, was collected by the writer at Lambton Mills, near Toronto, Ontario. Most of the specimens were collected during the summer and autumn of 1924, a small number in earlier seasons. Only those collections which have been actually utilized by the writer are listed

in the accompanying table. It will be noted that some of the species (e.g. *Hyalopsora*) were obtained at various dates throughout the season, whereas others were collected and, in fact, were available only within a narrow range of time. As indicated in the table, field collections were supplemented in some cases by material from inoculation experiments. In these experiments, aecidia from the alternate host were used as the source of inoculum.

Small pieces of leaves bearing uredosori were fixed in the field, chrom-acetic fixing fluid being used chiefly, picro-sublimate in a small number of cases. The material was then washed and passed through the alcohols in the usual way. It was transferred to the laboratory in 70 per cent. alcohol. After embedding in paraffin, sections were cut 5 to 10  $\mu$  in thickness. A number of stains were used, including Heidenhain's iron-alum-haematoxylin, Flemming's triple, safranin, eosin, erythrosin, and light green. Safranin and light green used in combination proved to be very satisfactory for the purposes of this investigation. The sections were stained for about 24 hours in a dilute aqueous solution of safranin and, after careful differentiation in the alcohols, were dehydrated and stained in a clove oil solution of light green.

For the study of germ pores in the uredospores, dried leaves carrying mature pustules were utilized. It was found that the germ pores were best demonstrated by boiling in lactic acid scrapings of the spores on a slide.

Table of Species investigated.

Species.	Host.	Date of Collection.	Spore Forms present.
<i>Hyalopsora Polypodii</i> , (Pers.) Magn.	<i>Cystopteris fragilis</i> , (L.) Bernh.	June 21	II
		" 24	II
		" 28	II
		Aug. 1	II
		Oct. 4	II
		Nov. 1	II
<i>Hyalopsora Aspidiotus</i> , (Pk.) Magn. = <i>H. Polypodii-Dryopteridis</i> , (Moug. et Nestl.) P. Magn.	<i>Phegopteris Dryopteris</i> , (L.) Fee.	June 21	III and II
		" 22	II
		" 25	III ,, II
		July 25	II
		Sept. 16	II
<i>Uredinopsis Atkinsonii</i> , Magn.	<i>Asplenium Filixfoemina</i> , (L.) Bernh. <sup>1</sup>	July 28	II
		" 29	II
<i>Uredinopsis Osmundae</i> , Magn.	<i>Osmunda Claytoniana</i> , L. <sup>1</sup>	Aug. 14	II
		" 26	III ,, II
<i>Uredinopsis Phegopteridis</i> , Arthur	<i>Phegopteris Dryopteris</i> , (L.) Fee.	Aug. 29	III ,, II
<i>Milesina polypodophila</i> , (Bell) Faull	<i>Polypodium vulgare</i> , L.	June 19	III ,, II
		" 22	III ,, II

<sup>1</sup> Material from inoculation experiments in which aecidiospores from the alternate host were used.

Species.	Host.	Date of Collection.	Spore Forms present.
<i>Milesina marginalis</i> , Faull and Watson	<i>Aspidium marginale</i> , (L.) Sw.	May 18	II
		June 16	III and II
		" 17	III " II
		" 19	III " II
		" 20	III " II
<i>Melampsorella elatina</i> , (A. and S.) Arthur = <i>M. Caryophyllacearum</i> , Schroet. = <i>M. Cerastii</i> , (Mart.) Schroet.	<i>Stellaria media</i> , (L.) Cyrill	July 14	II
	<i>Stellaria graminea</i> , L.	" 21	II
	<i>Stellaria graminea</i> <sup>1</sup>	" 26	II
	<i>Stellaria medi</i>	" 26	II
	<i>Cerastium vulgatum</i> , L. <sup>1</sup>	" 30	II
	<i>Stellaria medea</i> <sup>1</sup>	" 31	II
<i>Pucciniastrum Potentillae</i> , Kom.	<i>Potentilla tridentata</i> , Ait.	June 16	II
<i>Pucciniastrum arcticum</i> , (Lagerh.) Tranz.	<i>Rubus triflorus</i> , Richards	June 21	II
<i>Pucciniastrum pustulatum</i> , (Pers.) Diet. = <i>P. Epilobii</i> , Outh.	<i>Epilobium adenocaulon</i> , Haussk.	July 12	II
<i>Pucciniastrum Pyrolae</i> , (Pers.) Diet. = <i>P. Pirolae</i> , (Karst.) Schroet.	<i>Pyrola elliptica</i> , Nutt.	July 27	II
<i>Pucciniastrum americanum</i> , (Farl.) Arth. = <i>P. arcticum</i> var. <i>americanum</i> , Farl.	<i>Rubus idaeus</i> var. <i>aculeatissimus</i> , (C. A. Mey.) Regel and Tiling.	Sept. 16	III and II
<i>Thecopsora Vacciniorum</i> , Karst. = <i>Pucciniastrum Myrtilli</i> , (Schum.) Arth.	<i>Vaccinium canadense</i> , Kalm.	Sept. 8	II
	<i>Vaccinium pennsylvanicum</i> , Lam.	Sept. 16	III " II

## DEVELOPMENT OF THE UREDOSORUS.

*Hyalopsora*.

The genus *Hyalopsora* was established in 1901 by Magnus (33) to receive two species of *Melampsorella*—*M. Polypodii*, (Pers.) Magn., and *M. Aspidiotus*, (Pk.) Magn. These were held to differ from other species of *Melampsorella* in two features, namely, the absence of a peridium in the uredosorus and the occurrence of germ pores in the uredospores. Fischer (18) stated that the uredosorus of *Hyalopsora* lacks a peridium and that the uredospores are borne singly. Liro (28) claimed that a peridium is present in *H. Polypodii*, although absent in *H. Aspidiotus*, and on this basis he merged these species with those of the genus *Uredinopsis*. However, Magnus (36)

<sup>1</sup> Material from inoculation experiments in which aecidiospores from the alternate host were used.



reiterated his contention that there is no peridium in the uredosorus of *H. Polypodii*, but at its periphery a circle of paraphyses several layers in thickness. Lindau (26) and Grove (22) concurred with Magnus. Bartholomew (3) reported a peridium and stalked spores for *H. Polypodii*. Arthur (1) had previously described the spores as pedicellate, whereas Magnus and Grove reported them as sessile. Kursanov (24) described the formation of peridial cells in *H. Aspidiotus* and stated that the peridium becomes separated from the basal spore-forming cells by a dissolution of an intermediate 'disjunctive' layer. Lindfors (27) investigated the same species and found a delicate peridium and short-stalked spores. Bell (4) was inclined to regard the uredospores of *H. Aspidiotus* as sessile, pointing out that shrinkage due to desiccation or to poor fixation would cause the basal cells of the sorus to assume the appearance of specialized pedicels.

It is obvious, therefore, that the literature dealing with the uredosorus of *Hyalopsora* is very confusing. From it one might perhaps safely conclude that a peridium is present, and that the spores are borne singly and on pedicels. The latter point requires careful substantiation, however. Furthermore, although Kursanov describes the origin of peridial, disjunctive, and sporogenous cells, he does not explain how the spores arise from the latter. Also there seems to be some uncertainty regarding the precise mode of origin of the intercalary (disjunctive) cell. Kursanov (24) described the intercalary cell as arising by division of the subterminal cell of an erect hypha. This description was given for *Hyalopsora Aspidiotus*, and also for *Pucciniastrum Pyrolae*. In a more recent communication (25), without mentioning *Hyalopsora*, Kursanov gives a similar account for *P. Pyrolae*, namely, that the intercalary and sporogenous cells are sisters. Consequently, the development of the uredosorus of *Hyalopsora* is still imperfectly understood.

The thin-walled and thick-walled uredospores of *Hyalopsora* have been repeatedly a source of error for investigators. Schroeter (40) first called attention to these two types of spores, and tentatively suggested that those with thick walls are teleutospores. However, Dietel (10) germinated the latter, and showed that they behave in this respect like uredospores. Duggar (15) attempted to show that the thin-walled spores are an immature condition of the thick-walled spores—an erroneous conclusion. Arthur (1) regarded the thick-walled spores as aecidiospores. However, Bartholomew (3) found that the thick-walled spores arise from a binucleate mycelium, and concluded therefore that they cannot be aecidiospores as Arthur suggested. Grove (22) described the two kinds of uredospores as occurring at times in the same pustule. Fraser (20) stated that pustules with thick-walled spores are the first to appear in the spring. On the other hand, Dietel (12) said that the thin-walled spores come first in

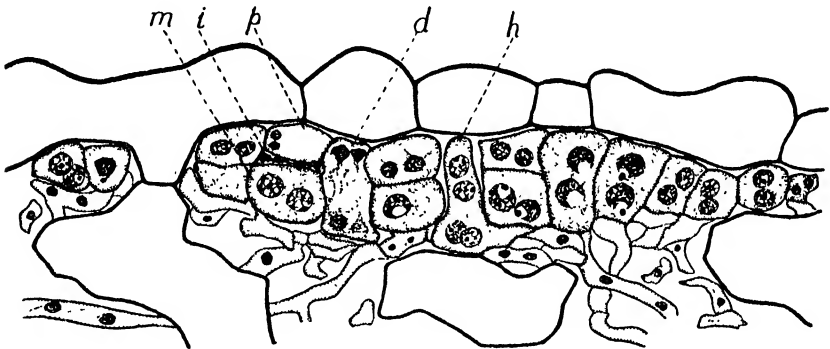
the spring, followed by the thick-walled; also that both kinds may be in the same sorus, the thick-walled proportionately increasing in the later-formed pustules.

My own observations on material collected from June 21 onwards support the conclusion that both kinds of spores may occur in the same sorus, and that thick-walled spores increase proportionately during the latter part of the season. The June collections of *H. Aspidiotus* showed many uredosori with thin-walled spores only, many with both kinds of spores intermixed, and several pustules containing only thick-walled spores. A September collection seemed to bear thick-walled spores only. Early summer collections of *H. Polypodii* carried a considerable proportion of thin-walled spores, although thick-walled spores were very abundant. The latter seemed to predominate in pustules on the rachis and older pinnae. There is considerable evidence that the first uredosori to appear on a young frond bear thin-walled spores, and that the latter may be succeeded by thick-walled spores arising from the floors of the mature and ruptured sori; also that uredosori arising on the older frond bear thick-walled spores at once. Indeed, it seems that production of thin-walled spores is not the expression of a seasonal periodicity inherent in the fungus, but, on the other hand, is correlated with the stage of maturity of host tissue. This is further evidenced by a collection made on October 4. Old yellowed fronds collected on that date carried thick-walled spores only; whereas fresh, young leaves (apparently very recently expanded and incidentally quite dwarfed) carried small uredosori which contained chiefly thin-walled spores.

Reverting to a general consideration of the uredosorus of *Hyalopsora*, my preparations show clearly that there is a peridium, that the spores are pedicellate, that the peridium becomes separated from the basal spore-forming cells by the disorganization of intercalary cells, and that the spore-initials arise as outgrowths of the basal cells. These features characterize the sori of both species examined. The following detailed account is applicable therefore to either species of *Hyalopsora* and to a sorus bearing either thin-walled or thick-walled spores.

The uredosorus begins as a rather loose hyphal plexus which, as far as my observations have gone, invariably arises close to a stoma. From this primordium, vertical hyphae arise, and expand greatly, the enlarged upper ends being cut off as cells. There is thus formed beneath the epidermis the 'assise en palissade' of Kursanov. Each cell of this palisade layer then divides by a horizontal wall to form two cells of about equal size. The lower is a sporogenous cell; the upper divides horizontally to form peridial and intercalary cells. A vertical section of a sorus at about this stage of development is shown in Text-fig. 1. The section is median and passes close to a stoma. The course of development outlined above tends

to proceed radially and in orderly fashion from the central part of the primordium. However, as may be observed in the drawing, symmetry does not always obtain, being interrupted, no doubt, by the peculiar conformation of the host tissue. In the central region of the sorus (Text-fig. 1) a somewhat belated hypha (*h*) has pushed through. In it, conjugate nuclear division for a sporogenous cell and its sister has taken place, but the cross-wall has not been formed as yet. A similar nuclear division has just been completed at *d*. Only one of the terminal cells has divided to form peridial (*p*) and intercalary (*i*) cells. The peridial is vacuolate and has small nuclei; the intercalary cell is very narrow and has degenerate nuclei. The latter cell has a very transitory existence, being crushed apparently by the upward expansion of the underlying sporogenous cell.

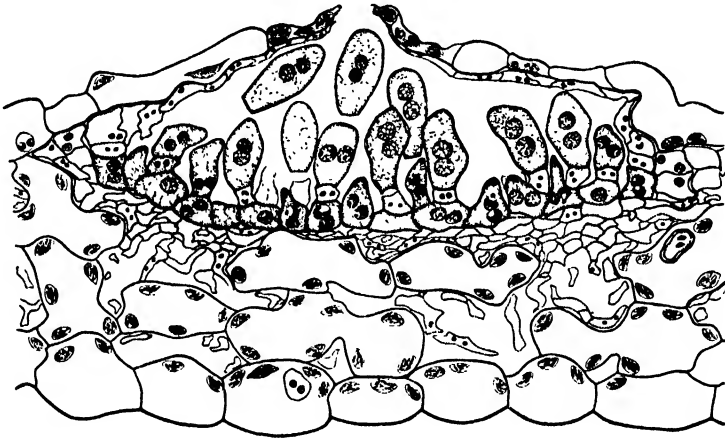


TEXT-FIG. 1. Median vertical section of a very young uredosorus of *Hyalopsora Aspidiotus*.  $\times 600$ . (*i*) intercalary cell; (*m*) sporogenous cell; (*p*) peridial cell. Further explanation in text.

Owing to the evanescent nature of the intercalary cell, difficulty has been experienced in reaching a conclusion regarding its origin. Kursanov stated that it is cut off from the sporogenous cell. Although quite impossible to prove that this never occurs, there is good reason to believe that peridial and intercalary cells are sisters. The peridial cell is characterized by a very large vacuole, by small nuclei, and by cytoplasm which stains faintly, whereas terminal cells of adjoining vertical columns in which intercalary cells have not yet appeared are less markedly vacuolate and have rather large nuclei and deeply staining cytoplasm. Evidently the terminal, not the penultimate, cell cuts off the intercalary cell. Unfortunately, I have been unable to find positive evidence of conjugate nuclear division associated with the formation of intercalary cells in *Hyalopsora*.

A spore initial arises as a bulbous outgrowth or 'bud' of the sporogenous cell. Conjugate nuclear division takes place at the base of the 'bud', which is then cut off by a transverse wall. The spore initial elongates and expands, its nuclei divide conjugately, and a rather short stalk-cell is cut off. At this time, a second 'bud' ordinarily arises from the

basal cell, growing out more or less laterally at first, but presently crowding upwards among the maturing spores. This 'bud' forms a spore as did the first. These stages are described and figured in greater detail under *Uredinopsis* and *Milesina*, whose uredosori develop in essentially the same manner. Upward growth of the spore initials destroys the intercalary cells and flattens the peridial cells. The latter adhere by their lateral walls and persist in the form of a thin peridium in close contact with the epidermis. The expanding spores exert pressure on the overlying host cells, flattening and eventually rupturing certain of them. Usually an irregular and a rather large opening is made, due, no doubt, to the delicate nature of the epidermal cells. The peridium persists beneath the epidermis as long as the latter is



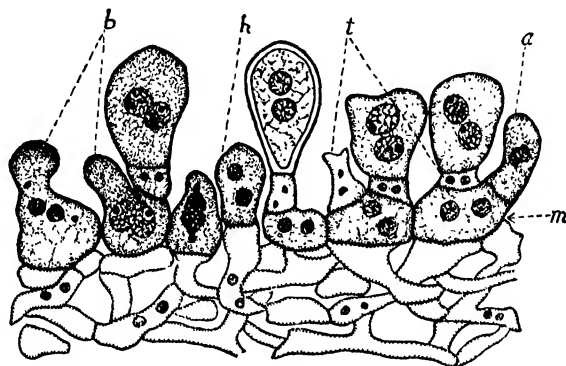
TEXT-FIG. 2. Median vertical section of a mature uredosorus of *Hyalopsora Polypodii*, showing various stages in the formation of spores.  $\times 370$ .

intact. In verification of this, photographs of rather old uredosori are submitted (Plate XXXIV, Figs. 1 and 2).

In Text-fig. 2 is shown a uredosorus from which spore discharge has recently commenced. Various stages in the formation of first and second spores from basal cells are to be observed. Extrusion of nucleoli, an early stage in nuclear division, is a conspicuous feature. Spore 'buds', spore initials, and remnants of pedicels from which spores have been detached are shown. At the periphery are columns consisting of basal, intercalary, and peridial cells or their progenitors. Presumably, these columns are the 'paraphyses' which Magnus described. There is an abnormal accumulation of starch grains in the injured epidermal cells surrounding the mouth of the pustule. Three haustoria are visible in the section, one of which is invested by a thick sheath. Germ pores are evident in some of the spores.

The uredosorus may expand radially until quite a broad pustule has been formed. Meanwhile, in the central region spore production continues.

There is good reason to believe that three or more spores may arise by a 'budding-out' process from a basal cell. However, it appears that these primary basal cells may become inactive—due perhaps to exhaustion or to pressure of underlying hyphae. In older pustules these cells become shrivelled and lose their affinity for dyes; in fact, frequently they are identifiable with difficulty. Spore production does not necessarily cease at this stage, however, for the hyphae of the underlying mycelial mat may force through, insinuating themselves among the original basal cells and cutting off spore initials. There is some evidence that these spore initials may become spores at once, that is, without cutting off stalks; in other

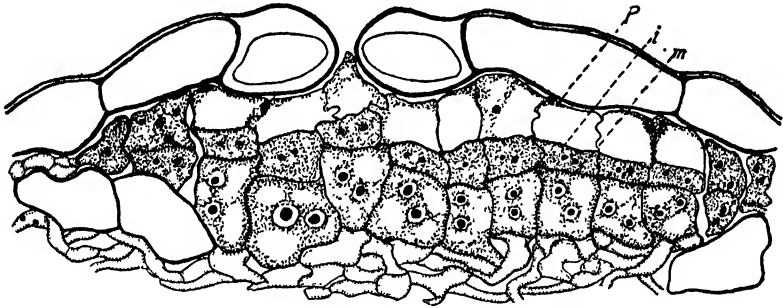


TEXT-FIG. 3. A group of cells from the floor of a mature uredosorus of *Hyalopsora Aspidiotus*. (a) spore initial; (b) spore 'buds' (one of which shows a stage in nuclear division); (h) spore initial cut off from a hypha that has pushed through the floor; (m) sporogenous cells; (t) stalk-cells (from one of which a spore has been detached).

words, it may be that, in old pustules, sessile spores occur occasionally. This process tends to lower the level of the spore-producing layer, and to bring about a sinking of the floor of the sorus into the tissues of the leaf, as is shown in Pl. XXXIV, Fig. 1. Of course, this is effected in part by the partial collapse of the underlying host cells. In Text-fig. 3 is represented a group of sporogenous cells from a mature sorus bearing thick-walled spores. There are five primary sporogenous cells, with spores and spore-initials in various stages of formation, and in one case a pedicel from which a spore has been detached. The middle sporogenous cell with the mature thick-walled spore is noticeably shrunk. In addition, there are two spore-initials cut off from hyphae which seemingly have pushed through from below. These 'spore-initials' may really be sporogenous cells which give rise to spores in the typical manner by a 'budding' process. However, as suggested above, such cells appear, in some cases at least, to become spores—stalked or possibly otherwise. In regard to pedicels, these may be quite short, even at maturity; or they may elongate considerably. Long stalks are encountered most frequently in deep-lying pustules on rachis and petiole.

*Uredinopsis*.

The literature dealing with the taxonomy, life-history, and morphology of *Uredinopsis* has been reviewed recently by Bell (4). It is apparent that the complex of rusts included under this generic name presents many problems still unsolved. One of the questions is that of dimorphism of uredospores. The earlier investigators, Dietel (11), Magnus (35), and Fischer (18), recognized two types of spores in addition to the so-called 'endospores' which were shown by Dietel to be teleutospores. Magnus claimed that these two types are uredospores, and intimated that they may occur in the same pustules. Fischer stated that they occur in the same pustule and that they intergrade. On the other hand, Fraser (20) noted

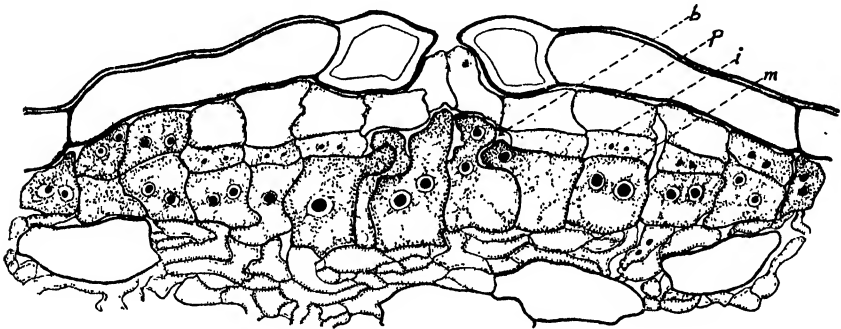


TEXT-FIG. 4. Median vertical section showing an early stage in development of the uredosorus of *Uredinopsis Osmundae*.  $\times 800$ . (*i*) intercalary cell; (*m*) sporogenous cell; (*p*) peridial cell.

that thick-walled spores were rarely present in his collections, and Bell (4) was able to locate only the appendaged fusiform type. Recently, however, I have examined collections of *U. Atkinsonii* from Timagami, Ontario, and found an abundance of thick-walled spores. It has so happened that in all my paraffin mounts I have found no positive evidence of thick-walled spores; consequently the subsequent description pertains to a uredosorus bearing thin-walled fusiform spores. It is fair to add that thick-walled spores have never been recorded for *U. Phlegopteridis* and *U. Osmundae*.

Another problem presented by the uredosorus of *Uredinopsis* is the mode of origin of spores and peridium. The spores have been regarded generally as occurring singly on stalks. However, recently, Bell (4) has claimed that the fusiform spores arise catenulately. Dietel described a peridium of elongated adhering cells, and Fischer stated that the peridial cells are elongated at the sides and in the upper part small and polygonal. Bell's illustrations of the uredosorus of *U. Phlegopteridis* represent a peridium, but do not show the elongated cells described by Dietel and Fischer. No account of the way in which the peridium develops has been found in the literature.

My study of the genus has failed to substantiate Bell's contention that the uredospores arise in chains. On the contrary, the evidence is conclusive that the spores are pedicellate and that they arise singly from basal cells by a 'budding out' process. A median vertical section of a very young uredosorus is represented in Text-fig. 4. Incidentally, it will be noted that the sorus originates beneath a stoma. In the central region, arising from the hyphal plexus, are vertical columns, each consisting of three cells. A study of the fate of the cells shows them to be peridial (*p*), intercalary or disjunctive (*i*), and sporogenous (*m*). An examination of the peripheral columns indicates that peridial and intercalary cells are sisters, also that the mother-cell of these is a sister of the sporogenous cell. For a comparison of a peridial cell with the terminal one of a two-celled column indicates that it



TEXT-FIG. 5. A stage slightly later than that shown in Text-fig. 4.  $\times 800$ . (*b*) spore 'bud'; (*i*) intercalary cell; (*m*) sporogenous cell; (*p*) peridial cell.

is the latter and not the basal cell which cuts off the intercalary cell. Conclusive evidence of this is the fact that signs of nuclear division have been observed in the terminal cell of a two-celled column. The peridial cells have degenerate nuclei and large vacuoles; the intercalary have small nuclei and cytoplasm which is uniformly distributed and which possesses a characteristic staining property with light green; the sporogenous are vacuolate, have huge nuclei with conspicuous nucleoli, and a cytoplasm that stains deeply with safranin. A slightly later stage in development is represented in Text-fig. 5. The sporogenous cells have grown upwards to form papillae (spore 'buds'), with the consequent destruction of the overlying intercalary cells and elevation of the central peridial cells.

Subsequent events are made clear by an examination of the peripheral region (Text-fig. 6) of a somewhat older uredosorus. The spore 'bud' (*b*) elongates and expands, and is cut off from the sporogenous cell by a transverse wall at its base. Presently this spore initial divides into a short stalk-cell (*z*) and an elongated pointed spore (*s*). These stages in spore formation are better shown in Text-fig. 7, a drawing of a group of cells from the floor of a mature sorus. It is clear that the spores arise from basal cells by

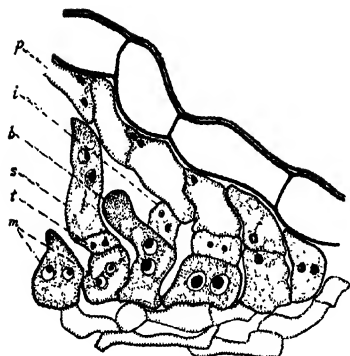


FIG. 6.

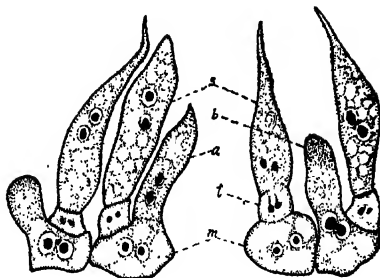


FIG. 7.

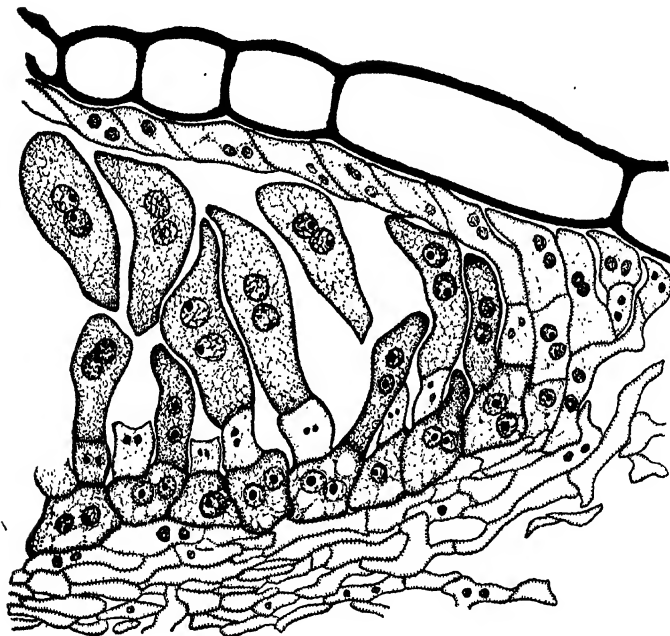


FIG. 8.

TEXT-FIG. 6. Peripheral region of a mature uredosorus of *Uredinopsis Osmundae*.  $\times 600$ . (b) spore 'bud'; (i) intercalary cell; (m) sporogenous cells; (p) peridium; (s) spore; (t) stalk-cell.

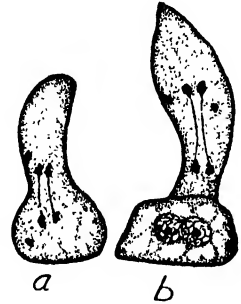
TEXT-FIG. 7. A group of cells from the floor of a mature uredosorus of *Uredinopsis Atkinsonii*.  $\times 600$ . (a) spore initial; (b) spore 'bud'; (m) sporogenous cells; (s) spores; (t) stalk-cell (remnants of spindles shown).

TEXT-FIG. 8. Half of a mature uredosorus of *Milesina polypodophila* in median vertical section, showing the mode of origin of peridium and spores.  $\times 600$ .



a 'budding-out' process. That several spores may originate from each basal cell is quite probable. Indications of conjugate nuclear division accompanying the process described above have been observed. The positions of spindles serve as evidence in corroboration of a series of events already outlined. Spindles associated with a similar course of development are represented (Text-fig. 9) for *Milesina polypodophila*.

Bell's drawings of older uredosori are excellent and accurate in every particular, although lacking in detail of peridium formation and 'budding out' of sporogenous cells. The latter cells are clearly represented, however, as also are the stalks which subtend the spores. Sporogenous and stalk cells were interpreted as immature spores by Bell, and hence he was led to conclude that the spores arise catenulately. His claim that maturing spores occur in chains, adhering end to end by their appendages, is most certainly erroneous. It should be intimated that in all three species of *Uredinopsis* studied by the writer, the uredosporos and peridia follow the same mode of development.



TEXT-FIG. 9. Conjugate nuclear divisions associated with spore formation in *Milesina polypodophila*.  $\times 600$ . (a) sporogenous\* cell bearing a spore 'bud'; (b) sporogenous cell with a spore initial.

### *Milesina*.

The early history of *Milesina* is bound up with that of *Melampsorella*. The genus was established by Magnus (35) to receive three species of *Melampsorella*, *M. Dieteliana*, *M. Kriegeriana*, and *M. Feurichii*. These were held to differ from other species of *Melampsorella* in that they possessed pedicellate uredosporos. Peridia were described for the uredosori of both genera, except for the species *M. Feurichii*, which was said to have a circle of paraphyses. Concerning the latter species, Magnus disagreed with Liro's (28) statement that a peridium is present and that the uredosporos are catenulate. Yet in his description of *Milesina* Magnus recognized a peridium. Three species of *Milesina* have been found in Ontario, one by Bell in 1923 (*M. polypodophila*), and two by Faull and Watson in 1924 (*M. Kriegeriana* and *M. marginalis*).

The two species of *Milesina* examined in this investigation exhibit rather striking morphological differences, and so will be considered separately. It will be noted, however, that the uredosori of these species follow the same mode of development.

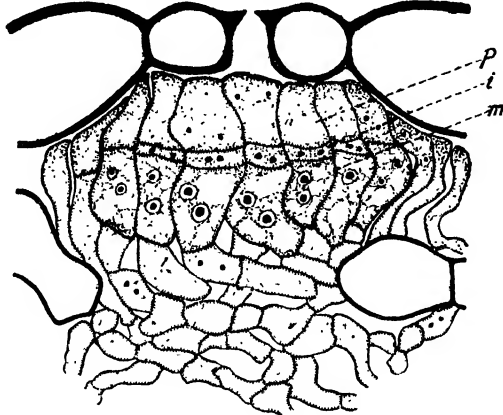
*Milesina polypodophila*. The species is described first because of the general resemblance of its uredosorus to that of *Uredinopsis*. With uredosori only as a clue, it is quite natural that Bell (4) was led to assign this

newly discovered form to the genus *Uredinopsis*. More recently the teleutospores have been discovered, and on account of their intradermal position the species has been transferred to the genus *Milesina* (Faull and Watson, 1925). The mature uredosori of *M. polypodophila* and *Uredinopsis* are similar in general shape and size (Pl. XXXIV, Fig. 9). Also the uredospores are somewhat similar, although those of *M. polypodophila* are not conspicuously appendaged, nor are they provided with longitudinal rows of delicate projections as in some species of *Uredinopsis*. The uredospores of *Milesina polypodophila* are smooth—an unusual feature for a *Milesina*.

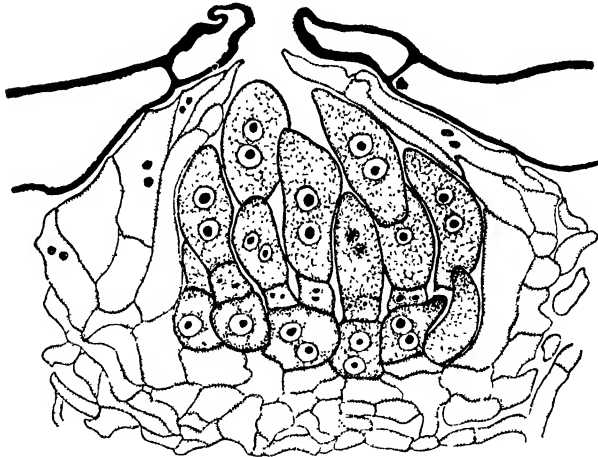
The uredosorus of this species, like that of *Uredinopsis*, commences below the epidermis as a rather extensive mycelial mat, which extends radially during development. Vertical columns arise, first from the central region, later from the outer parts of the primordium. Peridial, intercalary, and sporogenous cells are formed as in *Uredinopsis*, after which budding out of the sporogenous cells and the production of stalked spores ensues. Part of a mature sorus is represented in Text-fig. 8. The manner in which peridium and spores arise is quite apparent. Conjugate nuclear divisions associated with the production of spores were observed in various stages. As in other species studied, when a sporogenous cell pushes out a 'bud', its nuclei come to lie near the base of the 'bud' and there they divide. Nucleoli are extruded, spindles are formed which extend into the 'bud', and chromatin masses (or chromosomes) move apart on these spindles (Text-fig. 9). Presently a wall is formed across the base of the 'bud', which is now a spore initial. Following rapid expansion of the spore initial, its nuclei divide conjugately, the usual spindles being formed (Text-fig. 9), and a transverse wall separates stalk-cell and spore. The nuclear material which passes to the stalk-cell remains as a small mass which stains deeply, whereas that which goes to the spore loosens up and takes the form of a reticulum with which a nucleolus becomes associated and around which a membrane develops. The original nucleolus disappears during the nuclear division, or at least it gradually loses its affinity for safranin. The number of chromosomes appearing at nuclear division could not be determined with any degree of certainty in my preparations. It is worthy of note, however, that the general type of conjugate nuclear division in the *Pucciniastreae* is similar to that described by various investigators of other rusts.

*Milesina marginalis*. As noted above, Faull and Watson have recently found two species of *Milesina* which are new to Ontario. Although these rusts occur on ferns of the same genus (*Aspidium*), they have been shown by culture experiments to be distinct species (Faull and Watson, 17). Studies by Miss L. M. Hunter on the spermatogonia also show them to be distinct. The species on *Aspidium marginale* has been named *M. marginalis*, while that on *A. spinulosum* is tentatively regarded as *M. Kriegeriana*.

The primordium of the uredosorus of *M. marginalis* consists of a rather small mat of hyphae, substomatal in position. From this primordium stout hyphae arise and become divided transversely into peridial, intercalary, and sporogenous cells (Text-fig. 10), in the fashion already described for other



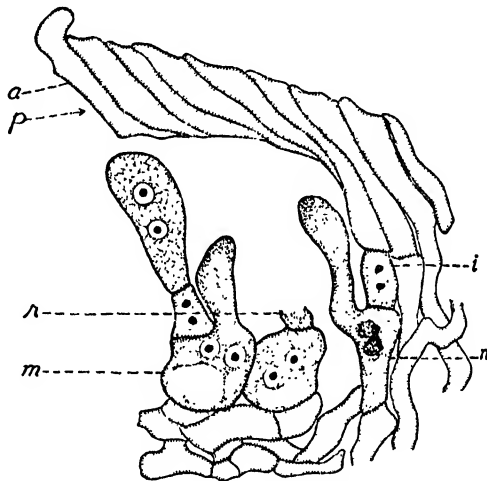
TEXT-FIG. 10. An early stage in development of the uredosorus of *Milesina marginalis*.  $\times 600$ .  
(*i*) intercalary cell; (*m*) sporogenous cell; (*p*) peridial cell.



TEXT-FIG. 11. A young uredosorus of *Milesina marginalis* in median vertical section.  $\times 600$ .

forms. Unlike *Hyalopsora*, *Uredinopsis*, and *M. polypodophila*, the original primordium of this species extends radially only to a very limited extent. Consequently, the width of the sorus never becomes great. Radial expansion is effected slowly by outward budding of the peripheral upright hyphae—a process which is initiated at an early stage. Budding out of the central sporogenous cells leads to the crushing of the intercalary cells, the elevation of the peridial cells, and the production of stalked spores. Mature spores

develop while the sorus is still quite narrow (Text-fig. 11). With the rupture of the central peridial cells, and the crushing of the guard cells, a narrow ostiolum arises, through which the spores escape. Additional spore 'buds' grow up from the central basal cells, others from newly formed sporogenous cells which arise peripherally. At the rather early stage represented in Text-fig. 11, the sorus appears to be surrounded by a bank of sterile cells, and it is not surprising that paraphyses have been described for this genus. However, these elongated hyphae, some of which are septated, are really destined to produce peridial, intercalary, and sporogenous cells. As already



TEXT-FIG. 12. Detail of a mature uredosorus of *Milesia marginalis*.  $\times 600$ . (a) ostiolar cell; (i) intercalary cell; (m) sporogenous cell, bearing a stalked spore and a spore 'bud'; (n) peripheral sporogenous cell, bearing a spore 'bud'; (p) peridium; (r) remnant of a stalk-cell.

indicated, some of these hyphae branch out centrifugally to form additional erect hyphae which add to the diameter of the sorus. The marked bending of hyphae and columns towards the stomatal opening is an interesting feature. A mature sorus is represented in Pl. XXXIV, Fig. 10, detail of which is shown in Text-fig. 12. The epidermis of the host is not elevated even by the mature pustule, and only a narrow orifice is made by the escaping spores. The way in which peridial and sporogenous cells continue to arise in the peripheral region is clear. Fundamentally, the development of this uredosorus is in agreement with that described above for other forms.

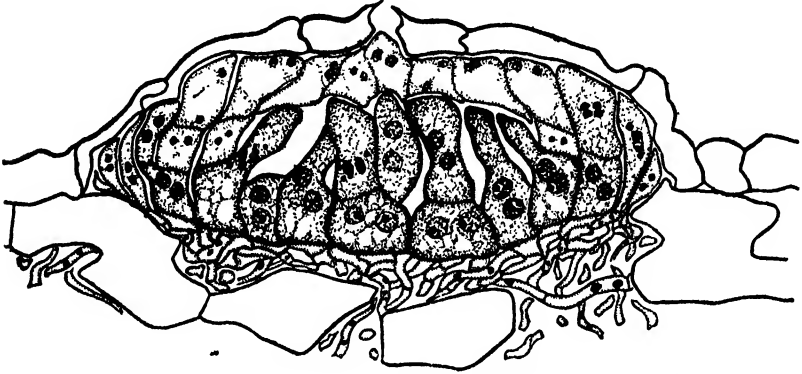
#### *Melampsorella.*

In establishing this genus, Schroeter (39) described a uredosorus with a peridium and sessile spores. Fischer (18) noted a peridium and that the uredosorus frequently arises under a stoma. Subsequent accounts agree

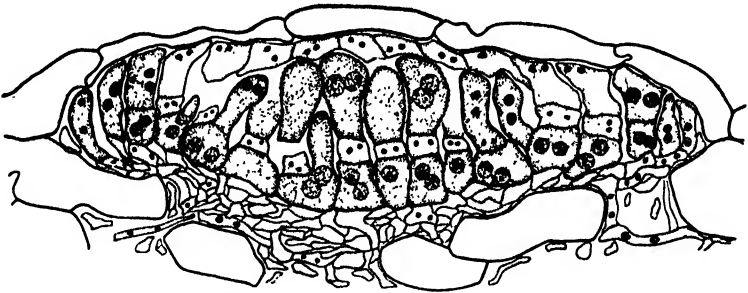
concerning the presence of a peridium, but are at variance regarding the way in which uredospores arise. Arthur (1) stated that the spores occur singly on pedicels. On the other hand, Liro (28) claimed that the spores are borne in chains. He points out that an examination of older pustules may give one the impression that the spores are borne singly on stalks, but that if thin sections of very young developing pustules be examined the fertile hyphae are seen to consist of three or four cells. He says further that the lowest or basal cell is somewhat irregular in that the side walls bulge out between the septa, that the middle cells are nearly cubical and slightly expanded towards the top, and that the apical cell is the largest, being nearly hemispherical on top and quite distinctly warted. Liro believes that this uredosorus has its counterpart in such rusts as *Chrysomyxa* and *Coleosporium*, in the first of which there is likewise a peridium. Magnus (36) confirmed Liro's assertion that the spores are catenulate. He made this claim for *M. elatina*—the species which Liro had examined. However, for the other species of the genus, *M. Symphyti*, (DC.) Bubak, he stated that the spores are usually formed singly, only rarely as many as two spores arising in a row. P. and H. Sydow (42), in their monograph, assert that the uredospores of *Melampsorella* are sparsely catenulate, the great majority occurring singly and without stalks. A review of the literature shows, therefore, that there is considerable difference of opinion concerning the uredospores of this genus. That disagreement should exist is not a matter of surprise to the writer, because in his experience the uredosorus of *Melampsorella* has proven quite difficult of interpretation, much more so than any of the others investigated.

In my investigation of *M. elatina* it was presently very clear that the uredospores arise, as a general rule at least, from basal sporogenous cells by a process of 'budding out'. Of interest here is Liro's observation that the lowest cells of the fertile hyphae are somewhat irregular in that the side walls bulge out; unquestionably, these irregular swellings are spore 'buds'. Furthermore, it was soon apparent, from a study of early stages, that the peridium consists of the terminal cells of erect hyphae, also that these cells are separated off by the disruption of subterminal 'disjunctive' cells. But it was not so easily decided whether spores arise catenulately between the 'disjunctive' cells and the basal sporogenous cells. In certain preparations there seemed to be evidence of as many as four cells in a vertical column—as Liro maintained—and if so, one of these cells was surely destined to become a sessile spore. Also in somewhat older preparations there frequently appeared to be a group of immature spores situated immediately below the peridium and above the basal cells, which at this stage were budding out. However, more careful study of a large number of preparations, showing various early stages in development, served to convince the writer that only three cells occur in a vertical column, these being peridial,

intercalary ('disjunctive'), and sporogenous cells. The appearance of as many as four cells in a column is believed to be a delusion, as also is the picture of a nest of sessile spores. Where a section cuts adjoining hyphae, the appearance of four-celled columns may be presented; this is frequently the case in tangential and in oblique sections. And where a section passes through a cluster of spore 'buds' these may be cut in such a way as to suggest immature spores, catenulately produced. It should be indicated



TEXT-FIG. 13. An immature uredosorus of *Melampsorella elatina* in median vertical section, showing peridial, intercalary, and sporogenous cells, and a budding out of the latter to form spore initials.  $\times 600$ .



TEXT-FIG. 14. A stage somewhat later than that shown in Text-fig. 13. Stalked spores have been formed and one sporogenous cell has developed a second spore 'bud'.  $\times 490$ .

here that the spore 'buds' may become quite elongated, radiating towards the stomatal region, where they sometimes become intimately associated with one another.

The accompanying drawings (Text-figs. 13 and 14) of medium vertical sections of uredosori show various stages in the formation of peridium and spores. Clearly the type of development is the same as in the forms described above. The fact that, in this species, the cells are quite small has made interpretation more difficult than in other cases. That peridial and intercalary cells are sisters is obvious, particularly since nuclear divisions in the mother-cell are observable. Pressure brought to bear by the expanding

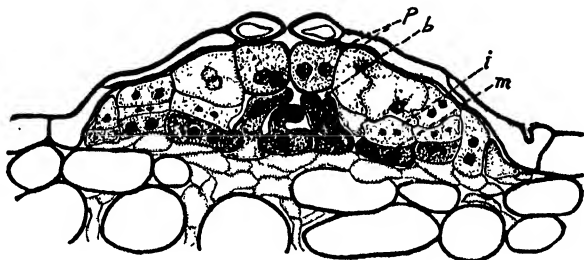
spore 'buds' brings about the disorganization of the intercalary cells and the elevation of the peridium. The cells of the latter are quite deep at an early stage, and later become greatly flattened. The spore 'bud' is cut off by a transverse septum to form a spore initial, the latter dividing into stalk and spore. The stalk may remain quite short or it may become considerably elongated. Subsequent budding of the basal cell results in the production of additional spores. As the central three-celled columns become disrupted by the expansion of spore initials new columns arise peripherally. Centrifugal expansion of the pustule is effected by the addition of erect hyphae which grow up from the underlying plexus or which bud out from young basal cells. The latter development is indicated at the left of Text-fig. 13.

A stage somewhat later than that represented in Text-fig. 14 is shown in Pl. XXXIV, Figs. 3, 4, and 5, which are sections from the same sorus. Certain features described above are more or less clear. In the peripheral regions, particularly of the tangential sections, may be seen something of a three- and four-celled catenulate formation; appearances of this kind probably led Liro and Magnus to conclude that the spores arise in chains.

#### *Pucciniastrum.*

In a careful description of the uredosorus of *Pucciniastrum*, Klebahn (23) indicated that there is a peridium, and that the spores are borne singly and on short stalks. Arthur (1) and P. and H. Sydow (42) gave a similar description. On the other hand, Ludwig and Rees (29), who made a critical study of *P. Agrimoniae*, asserted that the uredospores arise catenulately. The peridium was described as being made up of the terminal cells of vertical rows in the young sorus, the remaining cells of the rows becoming successively converted into spores. Disjunctive cells were not observed. The spore chains were said to arise from a layer of basal cells just above the hyphal plate. Colley (8) was led to question the conclusion of Ludwig and Rees concerning the catenulate origin of spores. He found, in his study of the uredosorus of *Cronartium ribicola*, that early stages corresponded closely with those figured by Ludwig and Rees for *P. Agrimoniae*. But, since the spores of *Cronartium* were found to be pedicellate and in no sense catenulately produced, Colley turned aside for a personal examination of *P. Agrimoniae*. His conclusion was 'that the method of formation of the urediniospores in *P. Agrimoniae* and *C. ribicola* is practically identical, and that therefore the spores in the uredinium of the former are not borne in chains but on stalks'. It is significant that Colley makes no mention of disjunctive cells for either *C. ribicola* or *P. Agrimoniae*; in the former species, the peridium is said to separate gradually from the underlying uredospores. Kursanov (24 and 25) described the early development of the uredosorus of *P. Pyrolae*. From

the primordium there develops a palisade layer whose terminal cells become peridial and whose subterminal cells divide to form disjunctive and spore mother-cells. The latter give rise to a succession of pedicellate spores, apparently by a budding-out process, although Kursanov is very brief in presentation of evidence. Dodge (14) has recently published an interesting paper on *Pucciniastrum*. In the young uredosorus of *P. americanum* vertical columns arise, the terminal cells of which constitute the peridium by adhering side by side. Subterminal or intercalary cells disintegrate, and in so doing liberate the peridium. Below the intercalary cells occur the basal sporogenous cells. It is stated that 'the first spore initials arise as a result of tangential divisions of basal cells, and not by budding'; subsequently, however, spore initials may bud off from the basal cells.<sup>1</sup> Uredosori of two other species, *P. Agrimoniae* and *P. Hydrangeae*, were examined. The



TEXT-FIG. 15. An immature uredosorus of *Pucciniastrum americanum* in median vertical section, showing (*b*) spore 'buds'; (*z*) intercalary cell; (*m*) sporogenous cell; (*p*) peridial cells.

former species resembles *P. americanum* in mode of development, whereas *P. Hydrangeae* shows little evidence of formation of uredospores by budding out of basal cells. It is to be noted that Dodge's account of *P. Agrimoniae* differs from that of Ludwig and Rees, as well as from that given by Colley.

My own observations on the uredosorus<sup>2</sup> of *P. americanum* are in general agreement with those of Dodge, with the exception that I find no evidence of spore initials being produced other than by a budding out of the basal cells. At an early stage in development there are observed upright rows, the central of which consist of three cells—peridial, intercalary, and sporogenous, as is indicated by their position, shape, and staining properties. A slightly later stage (Text-fig. 15) shows quite distinctly a budding of the basal cells, and a still more advanced stage shows the development of pedicellate spores from these buds. In mature uredosori (Pl. XXXIV, Fig. 7) it is evident that the basal cells continue to form spore initials by budding out.

<sup>1</sup> Dodge studied deep-seated sori on canes and petioles; hence his description is not necessarily applicable to the sori of the fungus on leaves.

<sup>2</sup> Sori on leaves only were examined.



Detail of the floor of a mature sorus is represented in Text-fig. 16. My preparations show, therefore, that the first spore initials to arise in the sorus are outgrowths of the basal cells. What happens in deep-lying sori I cannot state, as I have not studied pustules of this type.

The structure of the peridium of *P. americanum* is interesting. As explained by Dodge, the peridium becomes separated off by the disorganization of intercalary cells. Pressure brought to bear by maturing spores forces the peridium upwards, so that eventually the epidermis is thrown aside and the peridium stands out from the leaf as a long conical structure (Pl. XXXIV, Fig. 7). At an early stage, the central peridial cells show signs

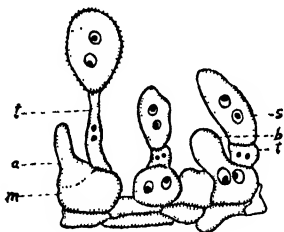


FIG. 16.



FIG. 17.

TEXT-FIG. 16. Detail of spore-bearing part of a mature uredosorus of *P. americanum*. (a) spore initial; (b) spore 'bud'; (m) sporogenous cell; (s) spore; (t) pedicels.

TEXT-FIG. 17. Upper part of peridium of *P. americanum*. Detail of uredosorus shown in Pl. XXXVI, Fig. 7.

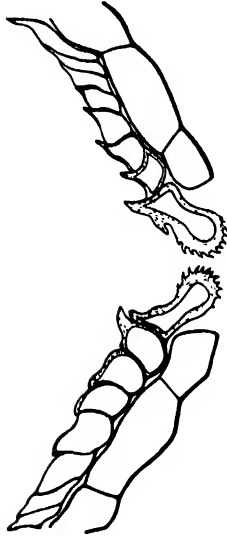
of becoming differentiated (Text-fig. 15). Compared with the adjoining cells, the central ones are not at all vacuolate, have nuclei and cytoplasm which stain deeply, and have walls slightly thickened. Eventually these cells become separated from one another and encircle the narrow ostium of the mature peridium. They are raised aloft as a result of the extensive lengthening, due to flattening, of the surrounding peridial cells (Text-fig. 17). The latter cells form the wall of the long conical peridium; at maturity they lose their contents, although degenerate nuclei may persist for some time and become somewhat thick-walled. The ostiolar cells become markedly differentiated—constricted near the base, very greatly thickened below, with recurved spines over the upper part. These observations on the ostiolar or corona cells are in accord with the original description by Farlow (16). On the other hand, Dodge contends that the so-called 'thickening' of the lower wall of the ostiolar cell is really a collapsed and disorganized condition of the cell. Dodge points out that the ostiolar cell is much shorter than the adjacent peridial cell, and argues that, since these cells were originally of the same length, the former must have become shortened by collapse and disorganization; this is said to have

occurred at the lower end. However, my preparations show that the cell subtending the ostiolar greatly exceeds its original length, whereas the ostiolar cell has been practically unaltered in this respect (Text-fig. 17).

In this connexion it is instructive to consider the uredoperidium of a closely related species, *P. arcticum*. The general habit of the uredosorus of this species is illustrated in Pl. XXXIV, Fig. 8. Detail of the peridium (Text-Fig. 18) shows that the cells become gradually thickened towards the

ostiolar region, and that the lower overlapping ends become sharp-pointed. The ostiolar cells are conspicuously aculeate and are greatly thickened, although more uniformly so than in *P. americanum*.

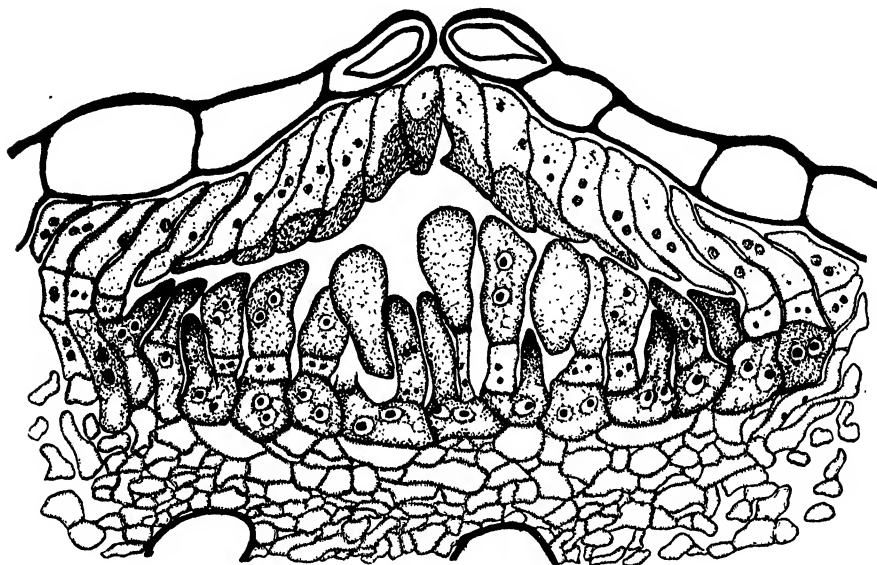
The peridial cells of *P. Pyrolae* are also greatly thickened (Text-fig. 19). Deposition commences at a rather early stage, and is at first confined to the lower end of the cell. Passing towards the ostiolar region, various stages in thickening may be followed. The protoplasmic contents persist in the cells to an advanced stage—apparently to the point at which deposition on the lateral walls commences. The substance laid down takes a deep safranin stain, and may resemble cutin in nature. As a bearing on an earlier discussion, it may be pointed out that in this peridium the peculiar appearance of the lower ends of the cells is a genuine thickening, and is certainly not due to a collapse and shrivelling; no shortening of the cell accompanies the alteration.



TEXT-FIG. 18. Detail of the uredoperidium of *Pucciniastrum arcticum*.

Reverting to a consideration of early stages in the development of peridium and uredospores, it has been found that all five species of *Pucciniastrum* examined are in these respects essentially alike. Those variations in development and structure which are exhibited by the uredosori of this genus pertain to extent and expansion of primordial plexus, to differentiation of peridial cells, and the like. Moreover, the course of events in the uredosorus of this genus is, in general, the same as described above for other genera. The mode of origin of peridium and uredospores of *P. americanum* has been described above. Early stages in development of the uredosorus of *P. arcticum* were not located; however, an examination of peripheral regions of older pustules indicates that the peridium originates in the typical manner, and, in older sori at least, the spores are stalked and arise by a budding out of basal cells. For *P. Potentillae*, early stages showing peridial, intercalary, and sporogenous cells were observed, as well as budding of the latter cells and subsequent events in spore formation. Very young uredosori of *P. Pyrolae* and *P. pustulatum* were not found. However, a stage such as that represented for *P. Pyrolae*

(Text-fig. 19) serves to indicate quite clearly the mode of development. Corresponding stages of *P. pustulatum* were observed, and were found to be very similar to those shown in Text-fig. 19. Of course, the peridium of *P. pustulatum* differs markedly from that of *P. Pyrolae*, in that the walls do not become thickened, nor the ostiolar cells greatly differentiated.



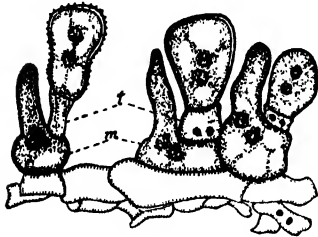
TEXT-FIG. 19. A rather young uredosorus of *Pucciniastrum Pyrolae* in median vertical section, showing mode of origin of peridium and spores.  $\times 600$ .

In Text-fig. 19 there will be observed in the peripheral region three-celled columns consisting of peridial, intercalary, and basal cells. Centripetal budding out of the latter, as well as subsequent stages in the formation of spores, are represented. In the central part, certain sporogenous cells have just matured their first spores, and second spore initials are developing. As noted above, Kursanov has recently made a study of the uredosorus of *P. Pyrolae*, and seems to have come to the same general conclusions as are herein recorded for this species. But in regard to the origin of the intercalary cell, I cannot agree with Kursanov's pronouncement on this point. Although I have not seen actual indications of division in the mother-cell, other observations and considerations point to the conclusion that peridial and intercalary cells are sisters.

### *Thecopsora*.

Some authorities do not recognize this genus as being distinct from *Pucciniastrum*. However, P. and H. Sydow (42) maintain the genus on the ground that the teleutospores are intradermal, whereas in *Pucciniastrum*

the teleutospores are intercellular and subepidermal. In other respects these genera are held to be quite similar. Pedicellate spores and a peridium are described for the uredosorus. In a paper already reviewed, Dodge (14) gives an account of a limited study of *Thecopsora Hydrangeae* (*Pucciniastrum Hydrangeae*). In the young uredosorus the spores are said to originate 'as a result of the cutting off of a spore initial from the basal cell as a whole, and not as a bud'. Dodge seems to conclude, therefore, that the spores arise catenulately in the young sorus. In the older sori, however, he says there is some evidence that the basal cell may bud out.



TEXT-FIG. 20. Detail of a mature uredosorus of *Thecopsora Vacciniorum*. (m) sporogenous cells, bearing stalked spores and spore 'buds'; (t) pedicels.

In material of *Thecopsora* studied by the writer, only mature uredosori have been found. An examination of these indicates that the peridium arises after the fashion described for *Pucciniastrum* and the other genera. The uredospores, in the older sorus at least, originate as in the other genera investigated. A mature pustule is shown in general outline (Pl. XXXIV, Fig. 6). Detail of the floor of such a pustule (Text-fig. 20) illustrates the mode of spore-formation, namely, by a budding out of the basal cells.

#### DISCUSSION.

*Development of Uredospores.* In all of the genera studied in this investigation, the uredospores are pedicellate and arise singly by a budding of basal cells. This is in disagreement with certain earlier accounts of *Melampsorella*, *Uredinopsis*, and *Pucciniastrum*, in which catenulate spores are described. As far as mode of development of uredospores is concerned, there is now no ground for differentiating the fern rusts of the Pucciniastraeae from those on Angiosperms. In regard to *Milesina*, which Magnus distinguished from *Melampsorella*, mainly in the belief that the uredospores of the latter are catenulate, the question now arises whether this genus should be retained. The answer seems to be in the affirmative, because, although fundamentally alike in regard to development of their uredosori, these genera differ in other respects, namely, the colour of uredospores and septation of teleutospores. Also, unpublished work of Professor Faull and Miss L. M. Hunter shows that the pycnidia of these genera are dissimilar.

The mode of spore-formation which characterizes the uredosori of the Pucciniastraeae is not confined to this group, but occurs in other sub-families of Melampsoraceae and in the Pucciniaceae. Sappin-Trouffy (38) described this method of spore-formation in the uredosori of *Uromyces*,

*Puccinia*, *Triphragmidium*, *Phragmidium*, *Melampsora*, and *Cronartium*. Sappin-Trouffy found, moreover, that the teleutospores of various members of the Pucciniaceae arise likewise by a budding of basal cells. More recently, Blackman (5), Christman (6), Dodge (13), Colley (8), Kursanov (24), and others have given similar accounts for various rusts akin to those described by Sappin-Trouffy. Of interest, too, is the fact that Christman (7) found a similar budding out of basal cells in the production of primary uredospores of *Phragmidium*. Therefore, this mode of spore development is not confined to any particular group of rusts or to any one kind of sorus. It appears to be a primitive rather than a recently specialized condition.

*The peridium.* The uredoperidium is made up of the terminal cells of three-celled columns which arise in the young sorus. The peridial cells adhere side by side, and are liberated from below by the disruption of intercalary (disjunctive) cells. Since the peridium arises in essentially the same way in all six genera examined, there is thus provided further evidence of the intimate relationship of fern and angiospermous rusts of the Pucciniastreae.

As far as I am aware, this particular mode of peridium formation has not been found elsewhere among the Uredinales. In other cases, where uredoperidia have been reported, there is no mention of disjunctive cells. For the uredosorus of *Cronartium*, Colley (8) has described a peridium composed of terminal cells, the subterminal cells in the young sorus being spore initials. However, Colley's illustration of a young uredosorus suggests the possibility of a different interpretation. Kursanov (24) has described a uredoperidium for *Chrysomyxa* (*Melampsoropsis*). The 'peridium' is loosely organized, and is stated to consist of two layers of cells, these being the terminal cells of spore chains. Disjunctive cells do not occur, although Kursanov suggests that the second layer may correspond to the disjunctive cells of *Hyalopsora*. Kursanov has also made the interesting discovery of a vestigial 'peridium' in the uredosorus of *Melampsora*, consisting in this case of a single layer of thin cells. As in *Chrysomyxa*, the 'peridial' cells are quite evanescent and do not adhere to form a protective covering; nor are disjunctive cells formed. Kursanov suggests that these 'peridial' cells function in creating space for the developing spores.

Of interest here is a feature described by Dodge (13) for the teleutosorus of *Gymnosporangium*. The basal cells from which the spore 'buds' arise are subterminal in position, while the terminal cells of the primordium expand and function as 'buffer' or space-forming cells. In mode of origin and in probable function, therefore, these 'buffer' cells of *Gymnosporangium* correspond closely to the 'peridial' cells of *Melampsora*. But whether the terminal cells of these two distantly related forms are to be considered homologous is questionable.

As Kursanov has pointed out, there now appears to be considerable uniformity in respect to uredo peridia among the Melampsoraceae. One hesitates, however, to regard these peridia as homologous structures throughout. There is perhaps reason to suppose that the peridial cells of the Pucciniastreae are the homologues of spores, and that the intercalary cells are the homologues of spore-stalks. Whether a comparison of this kind may be extended to the peridia of *Melampsora* and *Cronartium* is not clear at present. Concerning *Chrysomyxa*, Kursanov considers that the peridial cells have a morphological significance comparable with the peridial cells at the mouth of the aecidium. However, uredo and aecidial peridia are probably to be regarded as analogous rather than homologous structures.

Nevertheless, it is of interest to compare the uredoperidium of the Pucciniastreae with an aecidial peridium, such as described by Fromme (21). Correlated with the formation of a peridium in the aecidial stage, Fromme noted an extensive production of sterile cells external to the peridial cells. In the uredosorus under discussion, however, there are no sterile cells thus formed. As already suggested, the peridial cells of the uredosorus of the Pucciniastreae may be regarded as the homologues of spores, but whether they should be considered homologous with the peridial cells of the aecidium is questionable; certainly they and the intercalary cells are not homologous with the 'sterile tissue' of the aecidium.

#### GERM PORES OF UREDOSPORES.

Germ pores characterize the uredospores of the majority of rusts. In the Pucciniastreae, however, pores are described for the uredospores of only one genus, namely, *Hyalopsora*. For other genera of this family, pores are stated to be absent or are disposed of in non-committal terms such as 'indistinct', 'not discernible', 'not perceptible'. The genus *Uredinopsis*, for example, is generally said to be characterized by spores lacking pores. This is the usual statement in the monographs, and, Magnien (36) emphasized this as one of the distinguishing features between *Uredinopsis* and *Hyalopsora*. On the other hand, Fraser's (19) work on *Uredinopsis* seemed to indicate that pores do occur in the spores of this genus. He described the germination of the uredospores of *U. mirabilis* as follows: 'Germ tubes emerged from germ pores, two placed near the beak and two near the base of the spore.' Recently, Bell (4) has substantiated Fraser's account of the germination of *Uredinopsis* spores and has given figures of germinating spores which afford almost convincing evidence of the presence of pores.

In view of the unsatisfactory condition of our knowledge on this subject, I examined the uredospores of several representatives of the Pucciniastreae. Scrapings of spores were boiled in lactic acid on the slide. Chloral

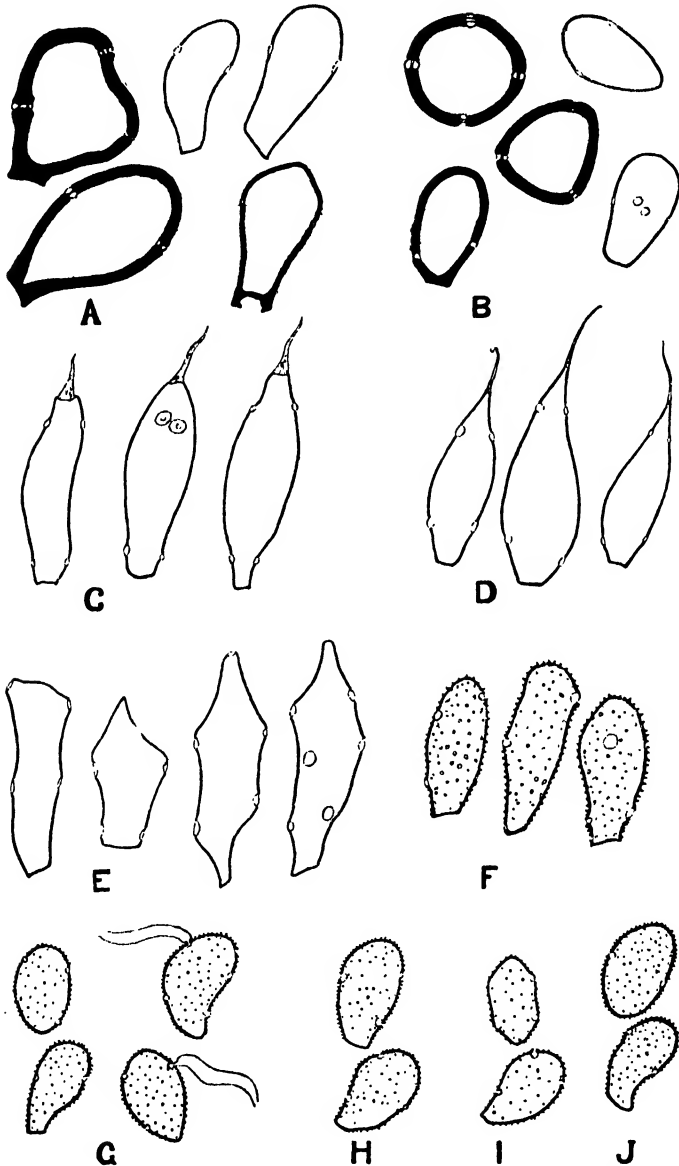
hydrate-iodine—cold and hot—was also used, but proved to be less useful than lactic acid. Boiling in chloral hydrate-iodine caused a bursting of the spores in some cases, whereas in lactic acid this did not appear to happen even upon prolonged heating.

The germ pores of *Hyalopsora* spores were found to answer the descriptions given in P. and H. Sydow's and other monographs. In the thick-walled spores the pores are quite conspicuous and may be seen in ordinary mounts. The pores of thin-walled spores are not so readily observed, but may be demonstrated by heating for about a minute in lactic acid. Spores of two species of *Uredinopsis* were treated in a similar manner and the pores rendered clearly observable. As indicated by Bell's drawings of germinating spores, the pores are four in number, two near each end of the spore. In the uredospores of *Milesina polypodophila* pores were likewise easily and distinctly demonstrated. The number of pores in this case appears to vary from four to six, depending on the size and shape of the spore. In longer spores there are two near each end as well as two in the equatorial region. For *Milesina marginalis* the pores were also clearly seen—only, however, after vigorous boiling in the acid. The number here appears to be four or fewer, and the distribution somewhat irregular—at times in opposite pairs, either towards the end of the spore or in the equatorial region. Observation of the pores in this species was rendered difficult by the tubercles on the surface of the spore. Pores were observed also in spores of *Pucciniastrum* and *Melampsorella*. However, prolonged boiling was required to make them evident, and in some cases careful examination at a high magnification was necessary to establish their identity with certainty. The pores are quite minute and are considerably obscured by the projections of the echinulate spore. Hence it is difficult to determine their number and distribution with certainty; but frequently there are four in the spore, two towards each end. The drawings (Text-fig. 21) represent spores as they appeared *in situ* under the microscope and do not show the total number of pores in every case.

These observations, therefore, point to the conclusion that germ pores characterize the uredospores of the *Pucciniastreae*. Moreover, for some genera at least, the pores are fairly constant in number and definite in position.

Absence of germ pores is generally regarded as a primitive condition, the emergence of germ tubes at definite and preformed parts of the spore-wall being considered an advance over the conidial type of germination. This is one of a number of primitive characters ascribed to the *Pucciniastreae*. Only one genus of the family has been thought to have pores in its uredospores, and that genus, *Hyalopsora*, has been considered an aberrant (Magnus, 86). However, in regard to germ pores there now appears to be no ground for considering *Hyalopsora* as being different from other genera

of the family. Nor, in this respect at least, can the Pucciniastreae be regarded as markedly primitive.



TEXT-FIG. 21. Germ pores shown in uredospores of: A. *Hyalopsora Aspidiotus*; B. *Hyalopsora Polypodii*; C. *Uredinopsis Osmundae*; D. *Uredinopsis Atkinsonii*; E. *Milesina polypodophila*; F. *Milesina marginalis*; G. *Pucciniastrum Potentillae*; H. *Pucciniastrum arcticum*; I. *Pucciniastrum pustulatum*; J. *Melampsorella elatina*.

It now appears that *Hyalopsora* and *Uredinopsis* are not unlike in those particular features by which Magnus originally distinguished these



genera, namely, absence of pores in the uredospores of *Uredinopsis* and absence of a uredoperidium in *Hyalopsora*. Germ pores and peridia are shown to be present in both of these genera. However, since these genera differ widely in other respects—colour and shape of uredospores, position of teleutospores, morphology of the pycnidium (Bell, 4)—there seems to be good reason for retaining the genus *Hyalopsora* as distinct from *Uredinopsis*.

### *Haustoria.*

Haustoria appeared so conspicuously in my preparations that I have been able to make a rather thorough comparative study of them throughout. In an investigation of this kind, one has in mind the possibility of locating features which are of taxonomic importance or which may be of phylogenetic significance; also one endeavours to gain an understanding of the physiological balance that exists between host and parasite in this very intimate relationship.

Haustoria have been described for a large number of the Uredinales. Sappin-Trouffy (37 and 38) established their general occurrence in the Pucciniaceae, as well as in *Melampsora*, *Coleosporium*, and *Cronartium*. He described also a species of *Melampsoridium*, one of the Pucciniastreae, under the name *Melampsora betulina*. According to his brief account, the haustorium of this species seems to resemble the haustoria of *Pucciniastrum*, described below. Among the various rusts which Sappin-Trouffy examined, the haustoria are quite variable in size and shape, ranging from simpler types to spiral, lobed, and branched forms. Also, there is exhibited a marked tendency on the part of haustoria to become associated with the host nucleus, sometimes entwining it and occasionally effecting certain deformations.

Although haustoria seem to characterize the Uredinales in general, they have been described for only a small number of the Pucciniastreae; indeed, they have been reported as not occurring in certain species of this sub-family. The present investigation indicates, however, that haustoria are present throughout the Pucciniastreae at least in the uredo host.

In every species examined the haustorium is typically binucleate. It is connected with a bulbous cell or appressorium by a very narrow tube which passes through the cell-wall of the host. The appressorium is cut off from a mycelial branch by a transverse septum. In most species a number of the haustoria are invested by thick sheaths or capsules. In staining properties the sheath resembles the cell-wall of the host, suggesting that it is composed largely of cellulose. Indeed, it appears that the sheath is laid down by the surrounding protoplasm of the host in response to the stimulatory action of the haustorium. Undoubtedly this constitutes

a defence on the part of the host-cell, because the encapsuled haustoria are in a disorganized condition. In certain species the haustoria are constantly associated with the host nuclei, whereas in others there is no evidence of a relationship of this kind. There is considerable variation in size and shape of haustoria among the forms examined. In the following descriptions the smaller and less elaborate types are considered first.

The haustorium of *Pucciniastrum* (Pl. XXXIV, Figs. 11–20) is irregularly cylindrical or allantoid in shape, and varies in size among the different species. In *P. americanum* it is quite small, being 10 microns or less in length, whereas in *P. pustulatum* the length is approximately 20 microns. As indicated in the drawings, a haustorium frequently becomes associated with the host nucleus, although disorganization of the latter has not been observed. Sheaths commonly develop around the haustoria, formation commencing at the base and proceeding towards the tip until finally the entire haustorium is enclosed. Whether the narrow tube at the base becomes closed during the process has not been determined. Encapsuled haustoria are shrivelled and obviously disorganized. As many as three haustoria have been observed in a single host-cell.

The haustorium of *Thecopsora Vacciniorum* (Pl. XXXIV, Fig. 21) resembles that of *Pucciniastrum* in structure. Intimate association with the host nucleus is a constant feature here. Moreover, the host nuclei are markedly affected by the fungus and undergo disorganization, a process which commences in the region adjacent to the haustorium. Host nuclei in various stages of degeneration were observed. Sheaths were noted in a small number of cases only.

For *Hyalopsora Polypodii* haustoria were reported by Magnus in 1892. In a later paper Magnus (31) stated that organs of this kind are absent in *H. Aspidiotus*. Unquestionably, however, haustoria do occur in both of these species, and moreover are quite conspicuous structures (Pl. XXXIV, Figs. 22–26). They are irregularly clavate or allantoid in shape and occasionally slightly lobed. In a small number of cases three nuclei were observed in the haustorium of *H. Aspidiotus*. Trinucleated haustoria were not found elsewhere. This is a phenomenon which occurs not uncommonly among the rusts, having been reported for spores in particular. In *H. Aspidiotus* sheaths appear to occur rarely and seem to resemble those of *Pucciniastrum* in nature. The sheaths of *H. Polypodii* are quite different, however, in that they are rather thin and are formed at a considerable distance from the haustorial wall. They resemble the wall of the host-cell in staining properties, and, indeed, are probably formed by the host protoplasm, which in this case has drawn away from the haustorium. These peculiar sheaths are of common occurrence in this species.

Haustroria have been observed in three species of *Uredinopsis*—*U. Osmundae*, *U. Atkinsonii*, and *U. Pheopteridis* (Pl. XXXIV, Figs. 27–30).

Magnus (30 and 35) has twice affirmed that haustoria do not occur in this genus. The haustorium is extensively lobed and branched at maturity, the processes being incurved or finger-like and more or less terminal in position. Sheaths were observed only occasionally.

In *Milesina marginalis* and *M. polypodophila* (Pl. XXXIV, Figs. 31–35) the haustoria are also elaborately branched, although here there is even greater irregularity in mode of branching, as well as greater diversity in habit, than in *Uredinopsis*. Early stages in development of sheaths were observed in the form of cup-like thickenings at the constricted bases of the haustoria. Incidentally, Magnus (33) stated that he could not locate haustoria in *M. Kriegeriana*, and in 1902 (34) he made the same statement for *M. Fenchii*. In the latter species, however, he observed 'Abheftungsscheiben', presumably appressoria, and suggested that these function as absorbing organs.

The remarkable haustoria of *Melampsorella elatina* were first described by de Bary (9) for the aecidial stage. In 1874 Schroeter (39) described the mycelium of the uredo stage, but was unable to locate haustoria, although he did note certain swellings on the hyphae and little hyphal brooms. Haustoria in the uredo host were first reported by Magnus (32). He noted the penetration of the host-cell by a narrow hypha and the extensive branching of the haustorium to form a series of brush-like structures within the cell. He described also the appressorium as a sort of pad on the outer part of the cell-wall. In my preparations I have found some haustoria which answer the description given by Magnus (Pl. XXXIV, Fig. 37). In other cases (Pl. XXXIV, Fig. 36) the haustoria are very similar to those of *Uredinopsis* and *Milesina*; in still other cases they are very elaborately branched and lobed, but at the same time compact in nature, and might well be described as botryose (Pl. XXXIV, Fig. 38).

Various stages in development of haustoria were observed (Pl. XXXIV, Figs. 39–42). Before penetration of a host-cell is effected a bulbous appressorial cell is cut off from the end of a hypha in contact with the host-cell. A very narrow tube from the appressorium then penetrates the host-wall and expands inside as a haustorium. The nuclei migrate through the narrow passage. There is no indication of a sheath appearing at an early stage in development, as has been described for haustoria of the Erysiphaceae. In the latter group of fungi, Smith (41) states that the sheath arises at the time of penetration of the host-wall by the haustorium. As Colley (8) has suggested for *Cronartium ribicola*, the sheath of the Pucciniastreae seems to be an accompaniment of maturity.

## SUMMARY.

The Pucciniastreae constitute a sub-family of the Melampsoraceae characterized by indehiscent teleutosori and aecidia of the peridermium type. Concerning the uredosorus of this sub-family, there has been considerable uncertainty and difference of opinion among investigators. Uredosori of fourteen species, representatives of six genera of the Pucciniastreae, have been studied by the writer.

Heretofore the uredospores have been described in three genera of this group as arising catenulately. This investigation shows, however, that the uredospores are pedicellate throughout, and that they bud out singly from a layer of sporogenous cells at the base of the sorus. This mode of spore development is not unlike that reported for the Pucciniaceae and certain other members of the Uredinales. Conjugate nuclear division associated with spore formation resembles that described for other rusts.

A uredoperidium occurs in all genera examined. It consists of the terminal cells of three-celled columns which arise in the young sorus. These terminal (peridial) cells are liberated by the disorganization of the subterminal or intercalary cells. The latter are disrupted by the upward growth of the first spore 'buds' from the basal sporogenous cells.

The peridium is compared with peridia which occur elsewhere among the Uredinales, and homologies are discussed. Peridial and intercalary cells are sister cells, and there is reason for regarding them as homologous with spore and spore-stalk, respectively.

Germ pores characterize the uredospores of the Pucciniastreae, and, for some genera at least, are fairly constant in number and definite in position.

Although *Melampsorella* and *Milesina* are not unlike in respect of development of their uredosori, they exhibit other characteristics which justify their retention as distinct genera.

*Hyalopsora* and *Uredinopsis* are not dissimilar in regard to those features by which Magnus originally differentiated these genera; however, their retention as distinct genera is justified on the basis of other differences.

Haustoria are of common occurrence throughout the group. They are similar in general organization, but vary greatly in size and shape, and exhibit certain generic and specific differences. Stages in penetration of the host-cell and in subsequent development are described.

On the basis of supposed differences in uredosori, certain earlier investigators have proposed a division of the Pucciniastreae into two groups, those on ferns and those on flowering plants. A general conclusion from the present work is that the uredo stage provides no criteria for a natural grouping of this kind.

This investigation was undertaken at the suggestion of Professor J. H. Faull, to whom I am greatly indebted for advice and assistance throughout. I am particularly grateful to Professor Faull and also to Messrs. W. R. Watson and G. D. Darker for an abundance of excellent material which they placed at my disposal. I wish also to acknowledge indebtedness to Miss L. M. Hunter for suggestions in regard to staining methods.

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## DESCRIPTION OF PLATE XXXIV.

Illustrating Mr. E. H. Moss's paper on the Uredo Stage of the Pucciniastreae.

Fig. 1. Mature uredosorus of *Hyalopsora Polypodii* in median section, showing peridium and 'sinking' of the spore-bearing region.  $\times 135$ .

Fig. 2. Mature uredosorus of *H. Aspidiotus* in median section.  $\times 135$ .

Figs. 3-5. Vertical sections of a uredosorus of *Melampsorella elatina*, Fig. 5 being almost median, the others tangential.  $\times 135$ .

Fig. 6. Mature uredosorus of *Thecopsora Vacciniorum*.  $\times 135$ .

Fig. 7. Mature uredosorus of *Pucciniastrum americanum*.  $\times 135$ .

Fig. 8. Mature uredosorus of *P. arcticum*.  $\times 135$ .

Fig. 9. Mature uredosorus of *Milestina polypodophila*.  $\times 135$ .

Fig. 10. Mature uredosorus of *M. marginalis*.  $\times 135$ .

Figs. 11-42. The haustoria are represented at a magnification of approximately 800 diameters.

Fig. 11. Haustoria of *Pucciniastrum americanum*, associated with host nuclei.

- Fig. 12. Haustorium of *P. americanum*, with a sheath at base.  
 Fig. 13. Haustorium of *P. arcticum*.  
 Fig. 14. Haustorium of *P. Pyrolae*.  
 Fig. 15. Haustorium of *P. Pyrolae*, with a sheath.  
 Fig. 16. Haustorium of *P. Potentillae*.  
 Fig. 17. Haustoria of *P. pustulatum*.  
 Fig. 18. Haustorium of *P. pustulatum*, with sheath partly developed.  
 Fig. 19. Haustorium of *P. pustulatum*, collapsed and invested by a sheath.  
 Fig. 20. Haustorium of *P. pustulatum*; similar to the last, but cut somewhat transversely.  
 Fig. 21. Haustorium of *Thecopsora Vacciniorum*, associated with a host nucleus, which is partly disorganized.  
 Fig. 22. Haustorium of *Hyalopsora Aspidiotus* (typical).  
 Fig. 23. Haustorium of *H. Aspidiotus*—trinucleate—an unusual condition.  
 Fig. 24. Haustorium of *H. Aspidiotus*, with a thick sheath.  
 Fig. 25. Haustorium of *H. Polypodii*, associated with a host nucleus.  
 Fig. 26. Haustoria of *H. Polypodii*, with thin sheaths which are formed at a considerable distance from the haustorial wall.  
 Fig. 27. Haustorium of *Uredinopsis Osmundae*.  
 Fig. 28. Haustorium of *U. Osmundae*, elaborately branched.  
 Fig. 29. Haustorium of *U. Osmundae*, encapsuled.  
 Fig. 30. Haustorium of *U. Atkinsonii*.  
 Fig. 31. Haustorium of *Milesina marginalis*.  
 Fig. 32. Haustorium of *M. marginalis*, with a collar at the base, presumably the beginning of a sheath.  
 Fig. 33. Haustorium of *M. polypodophila*.  
 Fig. 34. Haustorium of *M. polypodophila*.  
 Fig. 35. Haustorium of *M. polypodophila*, with a collar at base.  
 Fig. 36. Haustorium of *Melampsorella elatina*, simpler type.  
 Fig. 37. Haustorium of *M. elatina*, extensively branched type.  
 Fig. 38. Haustorium of *M. elatina*, botryose type.  
 Fig. 39. Early stage in formation of a haustorium of *Uredinopsis Osmundae* (prior to entrance into host cell).  
 Fig. 40. Later stage in formation of a haustorium. Young haustorium of *Pucciniastrum Pyrolae*, one nucleus having passed from the appressorium.  
 Fig. 41. A still later stage. Young haustorium of *Uredinopsis Osmundae*.  
 Fig. 42. Young haustorium of *Melampsorella elatina*.



1.



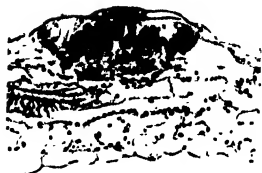
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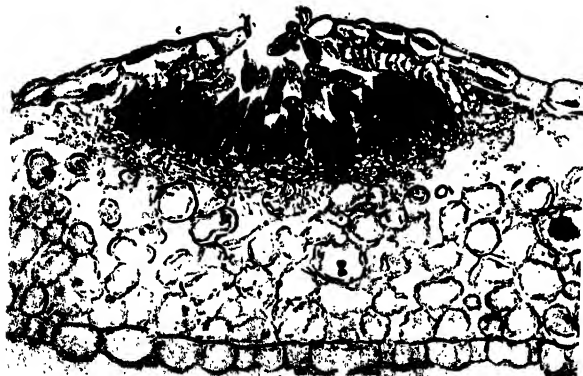
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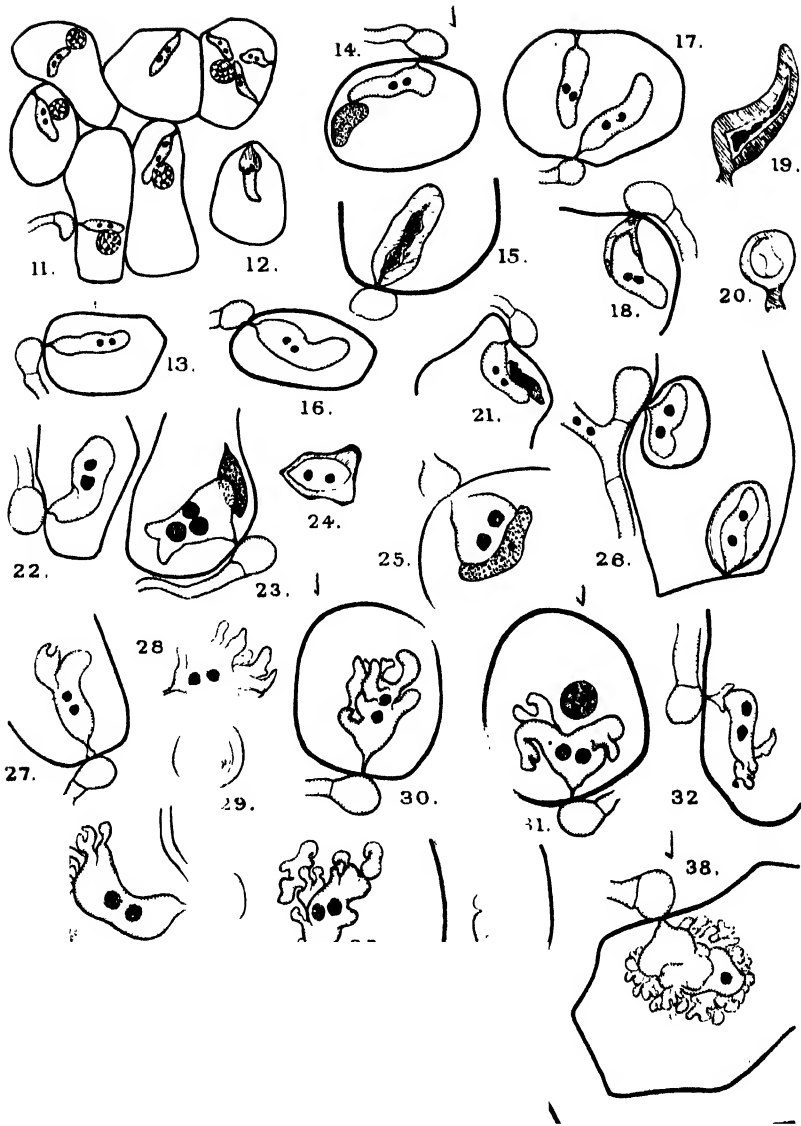
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10.









reported to be dioecious. In no case, however, was this statement confirmed by experimentation.

In the present paper the first case of heterothallism in the Oomycetes is reported,<sup>1</sup> together with numerous experimental data.

It is a pleasure to acknowledge the invaluable criticism and encouragement given the writer during this investigation by Dr. W. C. Coker, under whose direction the work has been done. To Miss Alma Holland the writer wishes to extend his sincerest thanks for inking in Plates III and IV.

#### HISTORICAL.

Fifty-five years have elapsed since the genus *Dictyuchus* was founded by Leitgeb on the single species *D. monosporus*. During this period four new species have been proposed: Lindstedt in 1872 added the two species *D. Magnusii* and *D. polysporus*; Zopf in 1893 described as new *D. carpophorus*; and Coker in 1923 gave specific rank to a sexually sterile form, calling it *D. sterile*.

The genus itself, though very clearly separated from its nearest relatives, *Thraustotheca* and *Aplanes*, is nevertheless considerably confused as regards the species composing it, as can be clearly shown by a brief survey of the literature on the subject. Leitgeb (8) in describing the species *D. monosporus* says the sporangia are renewed as in *Achlya*, his figures also showing this condition; he describes the oogonia as being  $25\mu$  thick, with antheridia which twine about them. Leitgeb also found a sterile species which bore only sporangia while under culture for four months. In *Dictyuchus Magnusii* the sporangia are described and figured by Lindstedt as being borne in rows; the oogonia are  $30-35\mu$  thick, and the antheridial branches do not wind about the oogonia. Lindstedt, in the same publication, described *D. polysporus*, in which the vegetative growth and sporangia resembled *D. monosporus*, but with numerous eggs ( $2-20$ ) in the oogonium, and with androgynous antheridia. These characters were so sharply in contrast with the single-egged oogonia and diclinous antheridia of the other two species that, taken together with the fact that the plant has not been found since first discovered, its validity as a species has been questioned by most subsequent observers, as Fischer (5), Minden (10), and Coker (4). It seems probable, as these authors suggest, that this species was no more than a sterile form of *Dictyuchus* mixed with an oogonia-bearing *Saprolegnia*. In 1893 Zopf (16) described as new a plant which he called *D. carpophorus*, the points of difference, according to his description, being in the peculiar lateral outgrowths of the hyphae, and in the fact that the oogonia, which contain

<sup>1</sup> First reported jointly by Dr. W. C. Coker and the writer before the Mycological Section of the Botanical Society of America, Cincinnati, Ohio, 1923.

a single eccentric egg, are often entirely encircled by antheridia which do not develop a fertilization tube. Coker (4), though not reducing any of the described species to synonymy, is of the opinion that *D. monosporus*, *D. Magnusii*, and *D. carpophorus* are the same. He is of the opinion, furthermore, that one of the two sterile plants reported by Humphrey (6) and the ones reported by Tiesenhausen (12), Minden (10), and Weston (15) are in all probability the same as his sterile species, *D. sterile*.

In addition to the confusion regarding species, there has also been a rather perplexing problem in regard to heterothallism in this genus. Leitgeb (8) and Lindstedt (9) both claimed that the antheridia and oogonia were borne on separate threads, and that their plants were therefore dioecious, a statement for which neither offers any cultural or experimental evidence. Neither Zopf (16) nor Humphrey mentions this matter. Weston (15) and Coker (4) suggest the possibility that the sterile form seen by them and others is either the antheridial or oogonial strain of a heterothallic plant.

#### VARIABILITY OF STRAINS IN NATURE.

In the summer of 1923 an excellent growth of *Dictyuchus* was found growing on a lightning bug in Lake Mendota, Madison, Wisconsin. This culture was brought back to the laboratory at Madison and kept under observation for several days. The sporangia were formed as usual in great abundance, and their shape, size, and method of renewal agreed well with *D. sterile*, but a few oogonia were developed which resembled those of *D. monosporus* as illustrated by Coker. A species of *Dictyuchus* producing oogonia had never been observed by the writer before, and so considerable care was taken to secure a pure culture. A few healthy-looking threads were cut from the growth and transferred to a fresh Petri dish of corn-meal agar. The plant grew well on the agar, forming a good many sporangia; but no signs of any sexual organs were observed. Transfers were made to boiled seeds of various kinds, as hempseed, corn grains, radish seed, &c., and cultured in sterile lake-water. Cultures were also made in haemoglobin, leucin, levulose, with and without the addition of various salts as used by Kauffman (7), Pieters (11), &c. Cultures were also kept in the constant temperature room at about 14° C.; but none of these cultural methods was successful in oogonial production. Numerous collections were made from the lake to secure the fruiting stage of the plant again, but without success. It was suspected that the plant might be an oogonial or antheridial strain of a heterothallic species, and so it was brought back to Chapel Hill, North Carolina, with a view to crossing it with *D. sterile*.

The plant, as it has been growing under culture, agrees in all details with *D. sterile* of Coker (4), and so the following, with slight modification, is taken from him: Vegetative growth vigorous on hempseed, corn

grains, &c., extending out a cm. or slightly more from the substratum; hyphae  $14-66\ \mu$  thick at base, usually about  $30-48\ \mu$ , more or less branched according to the cultural conditions. Primary sporangia apical, the later ones formed by cymose branching, but usually separated from the earlier ones by some distance by the elongation of the threads. As the culture ages, the arrangement becomes more irregular and complicated, and most of the threads become segmented towards the periphery into numerous sporangia arranged in rows or branched groups. The sporangia are usually a little larger in the distal half, often bent, sometimes branched, of various size,  $20-30 \times 100-550\ \mu$ . They generally break off from the hyphae about the time the outline of the spores becomes distinct, and going into a resting state which may last a few days or many weeks, depending on conditions. During this time the spores are separated by walls which, in this condition, are scarcely visible, the individuality of the spores being indicated by the usually conspicuous vacuole that each contains. On emerging, the spores escape and swim, as is normal in the genus, or they often sprout in position into slender hyphae. Spores  $11.8-16.6\ \mu$  in diameter, before sprouting, with a large conspicuous vacuole.

The Wisconsin strain, as mentioned above, was brought back to Chapel Hill with a view to crossing it with *D. sterile*. Numerous collections were made around Chapel Hill and, for the first time after fifteen years of collecting by Dr. Coker and his students, oogonia and antheridia appeared in three of the original lots of material brought in. The sexual organs appeared after the cultures had been in the laboratory about two weeks. One of these original lots of material, No. 1 of September 25, 1923, agreed best in most particulars with *D. Magnusii*, while the other, No. 1 of September 26, 1923, agreed best with *D. monosporus*. The former might be briefly characterized as follows: Hyphae stout, vigorous (on hemp-seed) as in the *Achlya racemosa* group, considerably branched. Sporangia usually long-cylindric, rarely short-clavate or oval, mostly  $300-400\ \mu$  long, not rarely up to 1 mm. long or slightly longer. Spores are characteristic of the genus. Oogonia spherical or slightly subspherical,  $35-52\ \mu$  thick, wall smooth, unpitted. Eggs single,  $30-34\ \mu$  thick, mature eggs eccentric. Antheridial branches of declinous origin; antheridia large, conspicuous, usually one or several on each oogonium, not rarely nearly completely wrapping around the oogonium, sometimes absent from an oogonium, in which case the oogonium may form a parthenogenetic egg. Antheridial tubes developed and clearly visible.

The present plant agrees well enough with *D. Magnusii*, as originally described by Lindstedt, in the size of the oogonia and eggs, but differs from that plant in the fact that the sporangia here are almost always borne in sympodia, while Lindstedt describes the sporangia in his plant as being in rows. The number of antheridia on the oogonia and their mode of

application are quite variable: the number varying from one to several, which may be applied laterally, or may completely encircle the oogonia. In order to distinguish this strain from the others it has been designated 'C'.

The second lot of material (collection No. 1 of September 26, 1923) contained a plant which agreed with the above in vegetative growth and sporangial characters, but differed from it in the size of the oogonia and eggs. The oogonia of the latter species measured  $27-33\mu$  thick, the eggs  $25-29\mu$ , eccentric, thus agreeing in this respect with *D. monosporus*. The antheridia varied from 1 to 5 on the oogonia, and might be tuberous and applied by their ends, or might be elongated finger-like and completely encircling the oogonia. The two plants could hardly be distinguished on the antheridial characters. This strain was designated 'B'.

A third lot of material was collected which contained antheridia and oogonia in the original culture. This plant might be characterized briefly as follows: Hyphae up to 1 cm. long, varying considerably in thickness. First sporangia formed in sympodia, the later ones formed in a row, sometimes as many as ten sporangia in a single row, quite variable in size and length,  $12-38 \times 100-200\mu$ , spores sometimes in a single row, sporangia deciduous. Oogonia  $27-33\mu$  thick, eggs  $22-29\mu$ , eccentric. Antheridia wrapped about the oogonia.

This plant agrees best with the description of *D. monosporus* and with strain 'B' above. It is noteworthy that the sporangia of this plant agree perfectly with those of *D. sterile*, and combine the characters of *D. monosporus* and *D. Magnusii*. This strain has been designated 'I'.

Numerous other sterile strains have appeared around Chapel Hill which, with but two exceptions, when grown unmixed with other strains of the opposite sex, agree with *D. sterile*. The thallic character of these strains as revealed by contrasting them with the oogonial and antheridial strains will be discussed at some length later on in this paper. One of the plants mentioned above which did not agree with *D. sterile* differed from it in that it combined the sporangial characters of *Dictyuchus* and *Thraustotheca*. The original culture contained many sporangia, the walls of which were bursting open and going to pieces, much as in *Thraustotheca*, and thus liberating the encysted spores. On the same threads with the *Thraustotheca*-like sporangia there could quite often be found sporangia of the *Dictyuchus* type. This strain has been designated 'G'.

Four other strains of *Dictyuchus* were collected during the last week in April, 1924, from near Georgetown, S.C., on the coast. Three of these strains, designated 'J', 'K', and 'L', differed in no particular from *D. sterile*. The other strain, designated 'M', which was bearing a large number of oogonia, was found growing on a mat of dying *Spirogyra* which had been parasitized by *Aphanomyces phycophilus*. The early sporangia of 'M'

differed in no way from those found in the original culture of strain B or C. The later ones, however, were quite irregular in shape, quite often antheridial threads or even antheridia being converted into sporangia, resembling very closely Zopf's figures of the sporangia of *D. carpophorus*. The oogonia were borne as in strain B and varied in size from 22 to 37  $\mu$ , the average of ten measurements being 30.3  $\mu$ , a good many proliferating and then without eggs, the eggs 18–32  $\mu$ , average of ten measurements 23  $\mu$ , eccentric. Antheridia on all oogonia, which contained an egg, simple or coiled around the oogonia.

During the latter part of June, 1924, the writer collected three strains of *Dictyuchus* in Florida. Two of these, designated 'O' and 'P', resembled *D. sterile* when grown separated from other strains. Their thallic character will be discussed later.

The other strain, designated 'N', collected in Florida differed from the other described strains in that it formed oogonia and eggs without any antheridia. The plant may be described as follows:

Vegetative growth and asexual reproduction as in *D. monosporus*, *D. sterile*, &c. Oogonia borne usually on threads which are considerably thinner and shorter than the main hyphae, and usually formed on the bottom of the culture, and hence often hard to see unless the culture is turned over; oogonial stalks commonly curved and often bearing several oogonia, 27–44  $\mu$  thick, most 35–39  $\mu$ ; usually spherical but sometimes slightly subspherical, smooth, and without pits. Eggs 23–35, most 29–33  $\mu$  thick, spherical, single in the oogonium, eccentric; antheridia not developed when the fungus is grown separate from other molds.

The present strain is easily distinguished from the others by its peculiar habit of forming oogonia and eggs without antheridia. The fact that the oogonia are borne on more or less specialized branches which usually grow out on the under side of the culture will also help to distinguish this plant. The oogonia, though formed in nearly all cultures, appear rather late, not before the fourth or fifth day, and never in large numbers. Some cultures, though quite vigorous and healthy, may bear only a single oogonial thread which in turn bears several oogonia. The results of crosses between this plant and other strains will be discussed farther on in this paper.

An effort was made to increase oogonial production by selecting the oogonial threads and making subcultures from them. This selection was carried only through a few generations; the cultures, however, showed no increase in oogonial production. Cultures on hempseed to which were added the solutions recommended by Kauffman (7) and Pieters (11) for oogonial production were made, but without any perceptible increase in the number of oogonia.<sup>1</sup>

<sup>1</sup> The writer has collected eight strains of *Dictyuchus* from different localities on Long Island, N.Y. All of these agree with *D. monosporus*, *D. sterile*, &c., in vegetative and asexual characters.



CROSSES TO DETERMINE THE THALLIC CHARACTER AND  
DISTRIBUTION OF THE STRAINS IN NATURE.

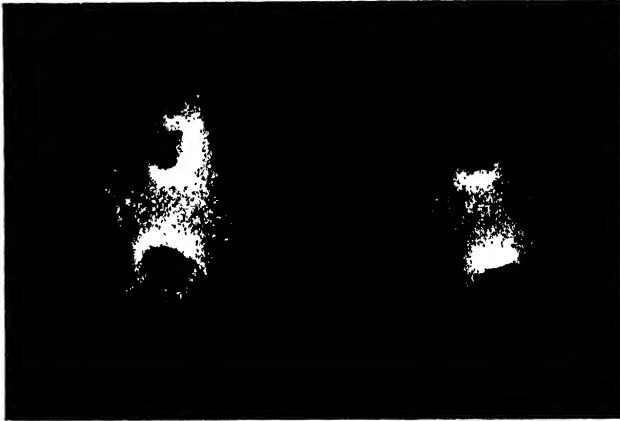
The original culture of strain 'B' contained an excellent lot of oogonia and antheridia. It was noticed that threads which bore oogonia never bore antheridia, and vice versa. But the oogonial threads occasionally bore at or near their tips sporangia, as did also the antheridial threads. It was hoped that by separating what seemed to be male and female parts and growing them alone, and then contrasting them on suitable media, the question as to whether the plant was heterothallic or not could be finally settled; and so a sporangium from an oogonial thread was carefully teased out under a low-power binocular with sharp needles and transferred to a corn-meal agar plate. This was also done with a sporangium from an antheridial thread. The strain descended from the sporangium cut from the oogonial hypha grew well on the corn-meal agar, forming sporangia but no oogonia. Cultures were made on oat-meal agar, small bits of hempseed, mushroom grubs, and on termites in sterile well-water, none of which, though the cultures were repeated many times, produced any oogonia, while sporangia were formed in great abundance. The same results were obtained with the strain descended from the antheridial sporangium. In the vegetative growth and sexual reproduction the strain descended from the female sporangium was indistinguishable from that descended from the male sporangium, there being no difference in vigour of growth as was described by Blakeslee (1) in the opposite strains in some of the heterothallic *Mucorineae*.

Unsuccessful in these attempts to induce the formation of oogonia by alteration in the nutritive medium, the male and female strains were grown together in the same dish with the mycelium of each strain partly in contact with that of the other. The methods pursued which gave the most satisfactory results were about as follows: Corn-meal agar cubes about 2-3 mm. thick were cut from the periphery of the circular area where the antheridial strain of the fungus was growing, so as to include in each of the cubes a good supply of the end region of the growing threads. Several of these cubes were placed in a sterile Petri dish, separated from each other and from the sides of the dish as far as possible. On each cube was placed a small piece of boiled hempseed (usually a half hempseed to each piece). To each of the incipient cultures was added a few drops of water. When the cultures were 24-48 hours old they were carefully washed by squirting a small stream of sterile well-water over them, and then fresh water was

Seven of them have, so far, given only neutral reactions with the stock male and female strains; the eighth is homothallic, but agrees in all other respects with the heterothallic strains. Detailed studies on these strains will be reported in a subsequent paper.

added. Cultures of the oogonial strain were made at the same time and in the same way, but were, of course, kept in separate dishes. When the cultures were about three days old an antheridial culture and an oogonial culture were transferred to a fresh Petri dish and placed so that the threads of the two strains were in contact. These cultures, unless otherwise noted, were kept at room temperature.

Two such crosses were made on October 20 between the antheridial and oogonial strain of B. These were examined three days later, and in the region where the threads of the two different strains were in contact



TEXT-FIG. 1. Natural size photograph showing two contrasts between male and female strains of *Dictyuchus*. The white specks in the regions where the threads intermingle are the sex organs. The elongated white specks scattered over the figure are sporangia which have become separated from the threads.

many oogonia and antheridia were formed, as is shown in the accompanying photograph (Text-fig. 1), while in the area where the threads were not in contact none was observed. Out on the margin of the region in contact where the hyphae were not so numerous and dense, the oogonial and antheridial threads could be traced back to the pieces of hempseed inoculated from the oogonial and antheridial strains respectively. Numerous contrasts were made in the same way as those above, except that an antheridial strain was contrasted with an antheridial and an oogonial with an oogonial, but in no such contrast were any oogonia or antheridia formed. From these two original oogonial and antheridial sporangia over two hundred cultures have now been made, and in none of these have any oogonia or antheridia appeared so long as they have been kept unmixed with the strain of the opposite sex. Over a hundred crosses have been made with these two strains, and almost invariably, when the cultures were young, healthy, and free from bacteria, oogonia and antheridia were formed in the region where the threads of the two opposite strains were in contact.

It is a noteworthy fact that in the later crosses between these strains the sexual reaction has become weaker; at present (March 1, 1924), oogonia are formed rather sparingly in the crosses and sometimes not at all. This gradual diminution in the ability to form sexual bodies was also found by Blakeslee (1) in a strain of *Mucor mucedo* when the cultures of the fungus were made from the mycelium.

In several of the crosses a rather peculiar and interesting variation appeared. The antheridia, which were quite variable even in the original lot of material, in these crosses, notably No. 45, were in many cases very elaborately developed, forming quite often a network around the oogonia several layers of threads thick. Even in these cases, however, one to several antheridia were cut off, antheridial tubes were developed, and fertilization apparently took place. These elaborately developed antheridial branches, twining about the oogonia so as to form a hull or network around them, were very suggestive of a similar condition described by Zopf in *D. carpophorus*, but his figures do not show the condition nearly so elaborate as it may be. In spite of Zopf's extensive description of his plant, some of the most critical diagnostic points, such as the size of the oogonia and eggs, are omitted, but from the facts at hand it seems probable that his plant is a variation of ours.

An effort was made to separate the two sexes in strain C, but unfortunately the male sporangium failed to grow.

In strain I the two sexes were successfully isolated in the same manner as described above for strain B. The first crosses were made on February 20. In five of these crosses both strains were growing on hempseed, in one the antheridial strain was on corn and the oogonial on hempseed, in two both strains were on corn. These were examined on February 22, and only in the two crosses on corn were any oogonia and antheridia formed. These crosses were examined again on February 29, but without any noteworthy change having taken place since the 22nd. Later crosses were made, but gave no sexual reaction at all. It seems that either the present strain is capable of giving only a weak sexual reaction, or that the proper environmental conditions for the production of oogonia and antheridia have not yet been afforded the plant.

The two sexual strains were isolated in strain M in the same manner as described above for strain B. The strains were cultured alone without the formation of any sexual organs, but when the two strains were grown in contact oogonia and antheridia were formed.

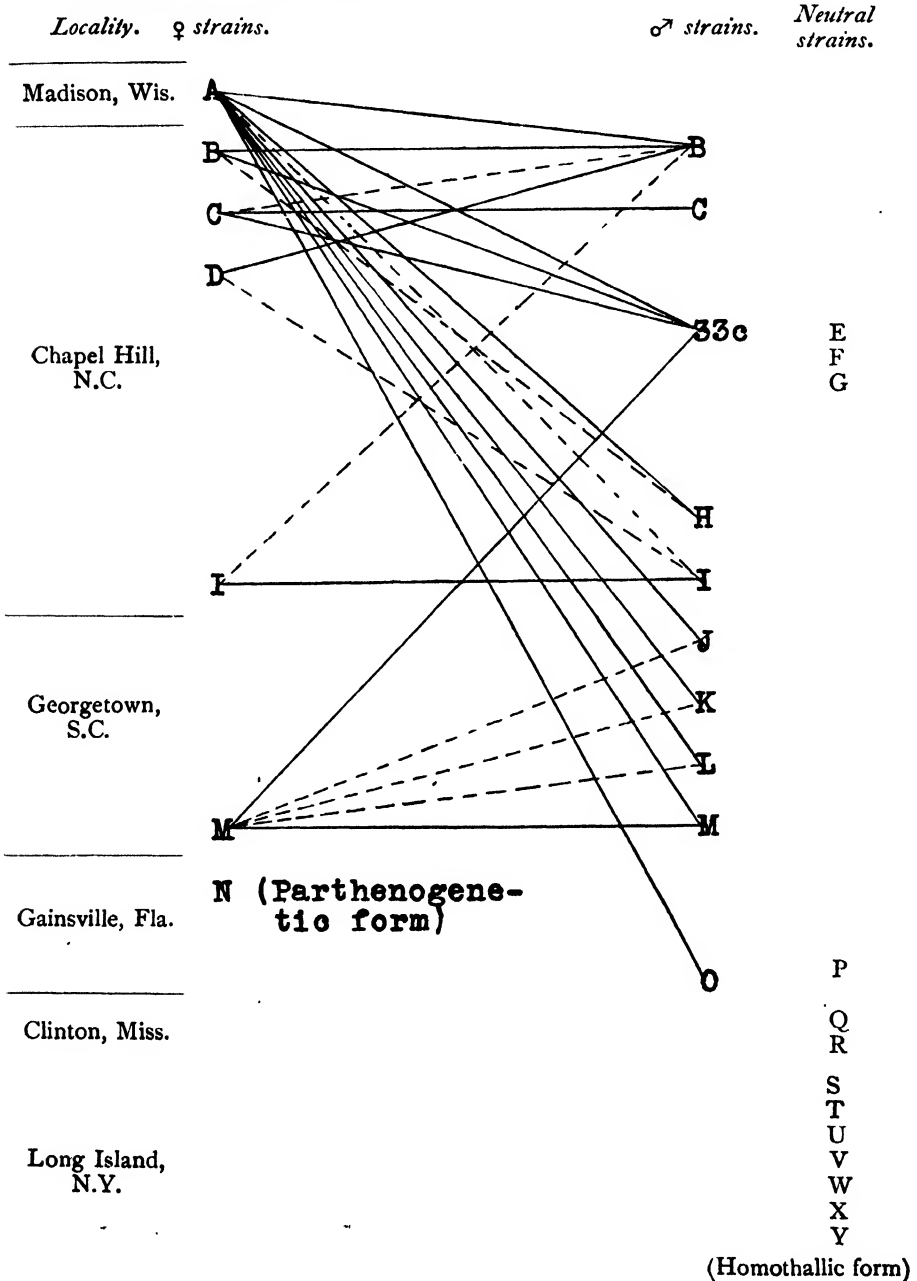
The results obtained by intercrossing the several strains brought to light some strikingly interesting information (see Table I). The first intercrossing was made with strain A from Wisconsin and both sexes of strain B from Chapel Hill. Numerous crosses were made between strain A and strain B, the cultures being grown on a considerable variety of media, as

pieces of boiled hempseed, corn grains, mushroom grubs, ant larvae, &c. Crosses were also kept in the oven with the temperature around 26° C. In none of the crosses, however, were any oogonia or antheridia produced. A healthy young culture of strain A, growing on a piece of hempseed, was crossed on November 1 with strain B ♂ growing on a piece of corn grain. The two cultures were placed so that the hyphal threads of each culture were in contact a short distance along the margin with the other. In this cross, three days later, a great many oogonia and antheridia were forming in the region where the threads were in contact. The oogonia were borne on threads which could be traced back to the hempseed, and the antheridial branches could be traced back to the corn grain. A good many oogonia were seen without antheridia, and in such cases failing to mature eggs. Over fifty crosses between strain A and strain B ♂ have now been made, and without exception, when the cultures were fairly free from bacteria, oogonia and antheridia were formed. The oogonial nature of strain A was thus established.

A comparison of the crosses between B ♀ and B ♂ and A ♀ and B ♂ reveals some interesting facts regarding the relative vigour of the oogonial strains in their reactions to the antheridial strain. As has already been noted above, in the first crosses between B ♀ and B ♂ many oogonia were produced, and in some of these crosses the antheridial branches were very elaborately developed. Gradually, however, the two strains seemed to lose their sexual vigour, and as the crosses were made fewer and fewer oogonia and antheridia were produced, until the present time (spring, 1924) only a small percentage of the crosses have any sexual organs, and these are sparingly formed. In the crosses between B ♂ and A ♀ the sexual reaction was very strong at first, the oogonia being formed before the antheridial branches became obvious, and in such abundance that many of the oogonia were not furnished with antheridia, and in such cases failed to mature eggs. The oogonial strain A seemed to be much more strongly stimulated than the antheridial, whereas in the crosses between B ♀ and B ♂ the antheridial strain seemed to be the one most stimulated.

Other experiments which indicate in a much more striking manner the relative strength of the oogonial strains when crossed with antheridial strain B were carried out. In one of these crosses the three strains were placed together in the same dish, and were arranged so that strain B ♂ was in the centre with strain B ♀ in contact on one side and strain A ♀ in contact on the opposite side. All the cultures were growing on hempseed, and were young, healthy, and practically free from bacteria. The crosses were examined eight days later, and many oogonia and antheridia were formed between A ♀ and B ♂, but none was formed between B ♀ and B ♂. Upon examination four days later, a few oogonia and antheridia were formed between B ♀ and B ♂. These crosses were repeated several times with

TABLE I. *Showing the Total Number of Female, Male, and Neutral Strains collected in Nature. The strains that crossed with each other are joined by a solid line. Sexual strains between which no reaction was obtained are joined by a dotted line.*



similar results, except that in a few of the crosses no sexual organs at all were formed between the strains B ♀ and B ♂. In another set of crosses the three strains were grown together in Petri dishes on corn-meal agar, made up according to the following formula: 3 grm. of agar shreds plus the filtered decoction from 20 grm. of corn-meal soaked in 500 c.c. of lukewarm water one hour; sterilized under 15 lb. pressure for thirty minutes in the autoclave. The agar plate was inoculated in the following manner: healthy threads were cut from a hempseed culture of strain B ♂ and placed approximately in a straight line in the centre of the dish, while threads were cut from strain A ♀ and B ♀ and placed on the plate in line with the threads of strain B ♂, but on opposite sides of the latter. The threads of the three strains were placed so that there was 0.8–1.0 mm. space between each line. The culture was examined two weeks after being made, and many oogonia and antheridia were formed between strain A ♀ and strain B ♂, but none was formed between strain B ♀ and B ♂. Similar crosses were made with practically the same results. The approximate sexual strength of the several strains may be judged from the data summarized in Table I.

It remained now to test the reaction of strain C, strains I ♀ and I ♂, and several other strains apparently identical with *D. sterile* with strain A ♀ and B ♂ and B ♀.

Numerous crosses were made between A ♀ and C, B ♂ and C, and B ♀ and C on different kinds of media and under different temperatures, but without the formation of any sexual bodies. Later, however, strain C was crossed with a strain 33 c ♂ (the origin of which will be explained farther on). This cross was made on February 11. Upon examination six days later, no sexual bodies had been formed. The culture was put in the oven at this time and examined again on February 22, at which time many oogonia and antheridia were formed. The thickness of ten oogonia, measured as they came into the field, varied from 34 to 48  $\mu$ , five of them being 37  $\mu$  thick; the ten eggs in these oogonia varied from 27 to 33  $\mu$ , five of them being 29  $\mu$  thick. There were one to several antheridia on each oogonium, at times completely covering the oogonium, in other cases applied only for a short distance along the oogonial wall. The oogonial nature of strain C was thus established, and also the identity of the plant which had been at first called *D. Magnusii* (strain C) with *D. monosporus* (strain B).

Several strains had been collected which in vegetative growth, sporangial characters, and the absence of sexual reproductive organs agreed with *D. sterile*. These were now crossed with the male and female strains to test their sexual reaction. Strain D, collected from the Arboretum Branch, January 10, and purified by the isolation of a single thread, was crossed on January 26 with strain A ♀, strain B ♀, and B ♂. Upon examination, January 29, no oogonia were observed. Strain B ♂, however, was branching considerably, forming what appeared to be antheridial branches. The cross

was examined again on February 7, but no oogonia had been formed. At this time the cultures were put in the oven, where the temperature was 26° C. Upon examination, four days later, a few oogonia had been formed between strain B ♂ and D. Several other similar crosses were made at the same time, and underwent the same conditions without the formation of any oogonia. It was suspected, therefore, that strain D was a mixed lot, partly neutral and partly weakly oogonial in nature, and an effort was now made to isolate the oogonial strain from the neutral strain. To do this, two sporangia from an oogonial thread were separated out and transferred to a corn-meal agar plate. Cultures descended from these sporangia were then made, and were contrasted on February 11 in the usual manner with the strain descended from sprouting egg 33 c, a very strong male strain. Upon examination, February 17, no oogonia were formed. The culture was then put in the oven, where the temperature was about 26° C.; it was examined again on February 29, but no oogonia had been formed. This cross was repeated several times with similar results. Crosses were then made with strain I ♂ and also with other strains, as strains G and F, but without the production of any sexual organs.

Strain E, also collected from the Arboretum Branch on January 10, was put through the same series of crosses as D, but without the formation of any sexual organs.

Strain F from Battle's Branch, January 16, which differed slightly from typical *D. sterile* in having considerably coarser hyphal threads and longer and larger sporangia, was crossed with strain A ♀ and B ♂, as described above, but without the appearance of any sexual organs. A cross on corn-meal agar was made in which threads of strain F were placed about the centre of the dish, with threads of strain A ♀ on one side and B ♀ on the other and B ♂ at one end, the three latter being arranged so that their threads would come in contact with those of strain F before they came in contact with each other. The threads of the latter three strains, however, showed an excellent growth in the direction away from F, but grew very poorly towards F, never coming in contact with F. Strain F grew very poorly itself except at the end away from strain B ♂, where its threads had a free range to the edge of the dish. Whether to consider strain F as a neutral strain or as a male or female strain of another species is a matter which can hardly be decided at present. It seems, however, that since both the antheridial and oogonial strains of *D. monosporus* are repelled by strain F the latter view is more probably correct.

A very peculiar strain G of *Dictyuchus* was collected from Howell's Branch on January 10. The original culture contained many sporangia, the walls of which were bursting open and going to pieces much as in *Thraustotheca*. The culture was purified by isolating on an agar plate a single sporangium of the *Thraustotheca* type from which the strain is descended.

Numerous contrasts were made in which antheridial strain B and an antheridial strain designated 116 (descended from a sprouting egg which was taken from cross No. 116 between B♀ and B♂) and strain A♀ and B♀ were used. In none of the crosses, however, was there any sign of oogonia.

Strains 'J', 'K', and 'L' from Georgetown, S.C., when unmixed with other strains, resembled *D. sterile*, were crossed with the stock male and female strains, but gave a reaction only with the female strain, thus proving them to be male in nature.

Strain 'O' from Florida was contrasted several times with the stock oogonial (A) and antheridial (33 c) strains, giving a strong reaction with the stock female strain, but none with the stock male strain, thus showing strain 'O' to be antheridial in nature. Strain 'P' from Florida gave no reaction in contrasts with either male or female strains.

Crosses were also made between the male and female strain of M and A♀ and 33 c ♂. The strain descended from the male sporangium designated M was crossed with A♀, many oogonia, eggs, and antheridia being produced. The oogonia varied in size from 37 to 55 $\mu$ , the average of twelve measurements being 43 $\mu$  thick; the eggs 29–44 $\mu$ , the average of the same number of measurements being 34 $\mu$ . A cross between the strain descended from the female sporangium M and strain 33 c ♂ produced oogonia, eggs, and antheridia much as in the above cross, but not in such abundance. Crosses between M♀ and M ♂ also gave a sexual reaction, as has been noted above, though it was considerably weaker than in either of the above crosses and the eggs failed to mature. It is interesting to note the remarkable variation in the size of the oogonia and eggs in the original culture of strain M as compared with the size in the cross with A♀ as shown in Table II.

In crosses which exhibited such remarkable variation in sexual activity one would reasonably expect considerable variation in the size of the oogonia and eggs, and also in the number of antheridia. As a matter of fact the oogonia and eggs showed considerable variation, but this seemed to be effected not only by contrasting a different oogonial strain with the antheridial, but also by altering the substratum on which the fungus was growing. The results in the following crosses represented in Table II will show the variation in the size of the oogonia and eggs. The oogonia in the above crosses show a total variation in thickness from 22 to 55 $\mu$ , the eggs from 18 to 44 $\mu$ , a variation certainly wide enough to discredit the validity of the two species *D. monosporus* and *D. Magnusii*. The antheridia varied from a simple, single, tuberos antheridium to a very elaborate network of threads completely surrounding the oogonium. The wide variations in the antheridia were quite difficult to explain, the variations occurring quite often in the same culture under apparently the same external conditions.

From the crosses described above it seems quite logical to conclude that *D. monosporus* and *D. Magnusii* are simply variations of the same species;



as is also, in all probability, *D. carpophorus*. The other forms which so far have failed to give any reaction with either the antheridial or oogonial strains may be unisexual or neutral strains of one or more entirely different species, or they may be neutral strains of *D. monosporus*.

TABLE II.

*Variation in Size of Oogonia and Eggs in Different Strains and Crosses.*

<i>Strains crossed.</i>	<i>Medium used.</i>	<i>Range in size of oogonia.</i>	<i>Average size of 10 oogonia.</i>	<i>Range in size of eggs.</i>	<i>Average size of 10 eggs.</i>
(1) Strain B, original culture	On mushroom grub in H <sub>2</sub> O, room T	27-33 μ		25-29 μ	
(2) Strain C, original culture	On corn grain in H <sub>2</sub> O, room T	35-52 μ		30-34 μ	
(3) Strain I, original culture	On corn grain in H <sub>2</sub> O, room T	27-33 μ		22-29 μ	
(4) Strain M, original culture	On dying spirogyra in H <sub>2</sub> O, room T, 22-24° C.	22-37 μ	30.3 μ	18-32 μ	23 μ
(5) B ♀ × B ♂	On hempseed in H <sub>2</sub> O, room T, 20-22° C.	32-44 μ	37 μ	25-34 μ	29 μ
(6) A ♀ × B ♂	On hempseed in H <sub>2</sub> O, room T, 20-22° C.	35-48 μ	40 μ	28-37 μ	33 μ
(7) A ♀ × B ♂	On hemp seed in H <sub>2</sub> O, room T, 26° C.	33-55 μ	42 μ	25-40 μ	33 μ
(8) A ♀ × B ♂	On corn-meal agar, room T, 20-22° C.	29-40 μ	33 μ	22-25 μ	23 μ
(9) C ♀ × 33 c ♂	On hempseed in H <sub>2</sub> O, room T, 26° C.	34-48 μ	35 μ	27-33 μ	29 μ
(10) A ♀ × M ♂	On hempseed in H <sub>2</sub> O, room T, 22-24° C.	37-55 μ	43 μ	29-44 μ	34 μ
		Total range, 22-55 μ		18-44 μ	

Numerous crosses have been made between the parthenogenetic strain and the male and female strains of *D. monosporus*. In twelve crosses between cultures descended from single spores from the parthenogenetic strain and strain A ♀ no reaction took place, the parthenogenetic strain forming oogonia and eggs as usual, while the female strain of *D. monosporus* was unaffected. Twelve crosses were also made between the spore strains and 33c ♂. In each case two cultures of the parthenogenetic strain were put in the dish, one in contact with the antheridial strain, the other one not in contact. On all the cultures of the parthenogenetic strain oogonia were formed as usual, and in one of the crosses a few antheridia were formed by 33c ♂ and were wrapped around the *Dictyuchus* oogonia.

In view of the fact that the parthenogenetic strain so closely resembled the other strains of *Dictyuchus* it seemed a matter of interest to germinate

the parthenogenetic eggs in the hope that the strains from the germinated eggs might throw some heterothallic varieties.

During the first part of January, 1925, four eggs were successfully isolated and germinated. A description of the methods used in egg germination is given farther on in this paper. These eggs were designated W, X, Y, and Z. The mycelium from sprouting egg W was divided into forty-nine parts, and each part was planted separately on a corn-meal agar plate; while the mycelia from sprouting eggs X, Y, and Z were transferred in entirety to separate corn-meal agar plates. After considerable growth had taken place, six cultures were made on hempseed in water from each of the forty-nine parts of W, and six each from X, Y, and Z.

Two contrasts were now made between each of the fifty-two strains (W 1-49, X, Y, and Z) and 33 c ♂ and A ♀.

In eighty-eight of the ninety-eight contrasts with 33 c ♂ and W 1-49 oogonia were formed on the strains descended from the parthenogenetic form, as usual when growing alone. In the remaining ten, no oogonia were seen on the parthenogenetic form, a fact of significance, however, only when these results are compared with the contrasts with A ♀, since some of the cultures of the parthenogenetic strain, when grown alone, form no oogonia. In sixteen of the ninety-eight contrasts the male strain, 33 c, was stimulated to the formation of antheridia which applied themselves to the walls of the oogonia of the parthenogenetic strain.

In each of the ninety-eight contrasts with A ♀ oogonia were formed on the cultures of W 1-49; in six of the contrasts very many oogonia were formed on W; in eight very few oogonia (not more than a dozen in each culture) were formed on W; while in the remainder from a few to a considerable number were formed on W. In two of the contrasts quite remarkable results were obtained. In these two not only did the parthenogenetic strain form oogonia, but A ♀ formed them also, and in many cases the latter were furnished with antheridia arising from the parthenogenetic strain, in which cases, as a rule, eggs were formed.

In the contrasts between eggs X, Y, and Z and 33 c ♂ oogonia were formed on the cultures of X and Z, but none were formed on the cultures of Y. No antheridia were seen.

In the contrasts between eggs X, Y, and Z and A ♀ oogonia were formed on all the cultures of the parthenogenetic strain, and in the contrasts between Y and A ♀ oogonia were formed on the latter and were furnished with antheridia which came from Y.

These experiments, though not succeeding in bringing out any heterothallic varieties of the parthenogenetic form, showed that the antheridial nature, though normally latent in the parthenogenetic form, was present nevertheless and could be brought out under certain conditions.

## CROSSES WITH OTHER MOULDS.

Blakeslee (1), in his work with the Mucorineae, was able to induce a process of imperfect hybridization by contrasting opposite strains of different heterothallic species in the same or even in different genera. It seemed a matter of considerable interest to make similar tests with the antheridial and oogonial strains of *Dictyuchus*. So far, contrasts have been made only with the genus *Thraustotheca*, the two species *T. clavata* and *T. primoachlya* being used with plus and minus strains of *Rhizopus* and *Phycomyces*. The two species of *Thraustotheca*, because of their close relation to *Dictyuchus*, offered especially good subjects for experimental crosses. Both species are homothallic plants, the antheridia of *T. clavata* being of diclinous origin while those of *T. primoachlya* are of androgynous origin. When well nourished, both plants produce abundant oogonia and antheridia in from three to five days. Several crosses have been made between *T. clavata* and strain A ♀ and B ♂, but without any apparent sexual stimulation on the part of either plant. The crosses between *T. primoachlya* produced considerable sexual stimulation. On February 12 a healthy two-day-old culture of *T. primoachlya* growing on a piece of hempseed in water was crossed with strain A ♀ and B ♂, the two strains of *Dictyuchus* being put on opposite sides of the culture of *Thraustotheca* so that there would be no chance of the two strains of *Dictyuchus* crossing with each other. The cross was examined four days later. The *Thraustotheca* had formed many oogonia over the entire culture, and many elaborately branched antheridial threads which, however, were much more elaborately formed on the side with strain A ♀ than on the opposite side. Between strain A ♀ and the *Thraustotheca* a few *Dictyuchus* oogonia were formed, which were borne on much-coiled and sometimes elaborately branched threads. None of the *Thraustotheca* antheridia were applied to the oogonia, which, as a probable consequence, failed to form any eggs. Between the strain B ♂ and the *Thraustotheca* there was no reaction. The culture was examined again a week later: the *Thraustotheca* had practically exhausted itself in the formation of sporangia and oogonia, but the two strains of *Dictyuchus* were still healthy and growing. And from some of the threads of strain B ♂ delicate antheridial threads had grown, extending across the *Thraustotheca* culture to apply themselves to oogonia formed by strain A ♀. It was a matter of considerable importance to determine whether the *Thraustotheca* had caused the stimulus in the two strains of *Dictyuchus*, or whether in some way the stimulus from the antheridial to the oogonial strain and vice versa had passed through the *Thraustotheca* threads. Accordingly the *Thraustotheca* was crossed with strain B ♂ alone on February 19. The culture was examined on February 21, but there was no obvious sexual reaction, nor was there any on February 25 or 29 or March 3. Two crosses of *Thraustotheca* with strain

A ♀ were made at the same time as the one described above. On February 21 *Dictyuchus* oogonia were forming in the region where the threads of the two fungi were in contact. The antheridial branches of the *Thraustotheca* were considerably stimulated on the side next to the *Dictyuchus*, but none was applied to the oogonia of the latter. Upon re-examining on February 25, 29, and March 3 none of the *Dictyuchus* oogonia was seen to be maturing eggs. The second cross was much as the above except that some *Thraustotheca* antheridia were apparently applied to the *Dictyuchus* oogonia. No eggs, however, matured in this cross.

All possible crosses were also made between the different sexual strains of *Dictyuchus* and plus and minus *Rhizopus* and plus and minus *Phycomyces*.<sup>1</sup> With these latter crosses corn-meal agar was used, a medium which would seem particularly adapted to these contrasts, since the opposite strains of *Rhizopus*, *Phycomyces*, and *Dictyuchus*, when crossed on it, produced abundant sexual reproductive bodies. One set of crosses was made on February 15 and kept out in room temperature. Several later examinations revealed no sexual response between the threads. (It was interesting to note that in all the crosses the threads of the two fungi did not repel each other but intermingled.) Another set of crosses was made on March 5 and kept in the incubator, where the temperature was about 26° C., with the same results as those just described above. It is quite possible, in spite of the above results, that a sexual response may be induced between strains of certain species of the Mucorineae and *Dictyuchus*, and it is hoped that a more exhaustive investigation may be carried out later.

#### EGG GERMINATIONS AND SEX DIFFERENTIATION.

The differentiation of this heterothallic species of *Dictyuchus* into antheridial and oogonial strains which, when cultured separately, are indistinguishable from each other, but which when crossed, even after the thirtieth non-sexual generation, produce sexual organs of such marked morphological distinctness as the antheridia and oogonia, offers a very striking example of heterothallism, for here in the fruiting condition the distinction between male and female is obvious, while in the Mucors no such distinction is possible, as Blakeslee (1) has shown. In *Dictyuchus* the germinating egg normally gives rise to a mycelium which may form one or several sporangia, and these in turn bear a large number of asexual swimming spores each of which is capable of growing into a new individual. It was, therefore, a matter of considerable interest to investigate the sexual nature of the mycelium and spores from sprouting eggs.

Germinating eggs of *Dictyuchus* have not been observed by previous

<sup>1</sup> The strains of *Rhizopus* and *Phycomyces* were kindly sent us by Dr. A. F. Blakeslee.

investigators. The writer was first able to induce germination after a resting period of six weeks to two months by placing ripe eggs in boiled corn-grain juice (made by boiling three or four corn grains about five minutes in 100 c.c. water) kept in the oven at a temperature of about 28° C., approximately the optimum temperature for growth—a scheme suggested by the work of Weston (14) in germinating the eggs of *Thraustotheca clavata*, though the latter investigator used pure water in the place of a nutrient solution. Later experimentation, however, has shown that the eggs will germinate in room temperature in boiled well-water.

The method used in germinating the eggs and separating them from the surrounding threads and resting sporangia was as follows: Part of a contrast in which there were about a dozen healthy ripe eggs was cut out with a scalpel and transferred to corn-grain juice. The eggs were observed daily under the low power of the microscope, and after three or four days the first visible signs of germination became evident. The protoplasmic contents of several of the eggs had swollen considerably, enclosing the oil globule and probably increasing in size at the expense of the latter. The egg-wall had become irregular on the inner surface, apparently being resorbed, this process continuing until the egg-wall had become comparatively thin. This swelling of the egg and resorption of the oil drop continued until the egg almost entirely filled the oogonium and until the oil drop almost completely disappeared. The egg was then ready to send out a germ-tube. When an egg had sprouted a short germ-tube, it was teased out under a binocular from the rest of the material along with its own oogonial stalk and old adhering antheridial branches and transferred to a fresh drop of nutrient fluid. Here, under the binocular, with fine glass needles the antheridial threads, which, however, were apparently dead, were torn off. In some cases, however, it was impossible to separate the antheridia from the oogonia. The germinating egg was carefully observed in order to be sure that nothing but the egg was sprouting. After the germ-tube had grown into a slightly branched mycelium, the egg with its germ mycelium was either transferred to a fresh agar plate or a fresh large drop of nutrient fluid was added.

The tests of the germinating eggs fall into two classes: tests of the sexual nature of the mycelium directly descended from the sprouting egg, and tests of the sexual nature of the mycelium descended from a single spore which was descended from a sprouting egg.

Tests of the mycelium directly descended from the sprouting eggs will be described first.

Ripe eggs were cut from cross No. 33, which was made between B ♀ and B ♂ and in which oogonia were formed on November 28, and put in boiled corn-grain juice at a temperature of about 28° C., on January 21. Five days later several of the eggs had sprouted. Five of these sprouting eggs

were separated from the threads, &c., and separately planted on fresh corn-meal agar plates. The strains descended from these sprouting eggs have been designated 33 a–33 e. One of the strains, 33 b, seemed to have been injured in transference and failed to grow; another one, 33 d, gave origin to a sporangium containing seven spores, three of which germinated (see Pl. XXXVIII, Fig. 9). The sexual nature of these spores will be described later.

Two crosses were then made between each of the strains to be tested and the stock male and female strains. In none of the six crosses between the stock male strain B and the strains tested were any oogonia formed. In several of these crosses threads which had the appearance of antheridial branches were considerably developed, especially in the region between the different strains, being often apparently attracted by each other, coming in contact and apparently anastomosing in places. In all of the crosses between the stock female strain A and the strains tested oogonia were formed. In the series of crosses between A ♀ and 33 a but few oogonia were produced, while in both of the other two series of crosses between A ♀ and 33 c and A ♀ and 33 c the sexual reaction was quite strong. In the first of these last two, many oogonia were formed, the oogonia proliferating abundantly; the antheridia, however, were rather sparingly developed. In the second, A ♀ × 33 c, oogonia were produced in great abundance, many more than in other crosses, being formed over the entire female culture, but only sparingly on the sides away from 33 c. The oogonia formed on the sides farthest away from the male plant proliferated considerably, until they were reached by antheridial branches which grew directly to them for a distance of about 2 mm. The oogonia on the sides were formed several hours before any antheridia were in their vicinity; none, however, were seen which had formed eggs, whereas many of those in between the cultures, where the antheridial branches were numerous, had formed eggs. The antheridial branches were also abundantly developed, showing in a few cases the remarkable peculiarity of swelling considerably at the tip as though beginning to form an oogonial initial.

At the same time as the above eggs were sprouted and their sexual nature determined, several eggs from a cross between B ♀ and B ♂ (cross 116) were sprouted and tests were made of their sexual nature. Of several eggs which sprouted, however, only one grew (designated 116 d). Numerous crosses were made as usual with parts of the mycelium from this egg and the stock male and female strains. In all of the crosses between 116 d and A ♀ oogonia were formed, while in none of the crosses between the sprouting egg strain and B ♂ were any oogonia produced, thus indicating the male sexual nature of the mycelium from this sprouting egg.

The evidence from the above germinations and crosses seemed to indicate that sexual differentiation took place at an early stage in the egg

germination. It also has indicated, in the case of sprouting egg No. 33 c, that the sex differentiation remained constant after once being determined. The significance of this statement can be grasped only by recalling the way in which the fungus is cultured and mycelial transfers are made. Stock cultures of the fungus are kept growing on corn-meal agar plates in Petri dishes. When the mycelium has about covered the plate, as it usually does in about two weeks, it must be transferred to a fresh plate. This is done by cutting out a small cube of the agar with some of the growing ends of the fungus and planting this cube on a fresh agar plate. Hempseed cultures for crosses are inoculated in essentially the same way. It is obvious, therefore, that only a very small fraction of the mycelium is tested, and that if sexual differentiation should take place in the mycelium a good while after the egg had germinated it would easily be possible, in making the transfers, to miss one of the sexual strains entirely. To lessen the possibility of such an occurrence in the cultures of 33 c, the mycelium used for inoculation was cut from several different spots in the agar culture, and yet the crosses with A ♀ or other female strains continued to give a strong sexual reaction.

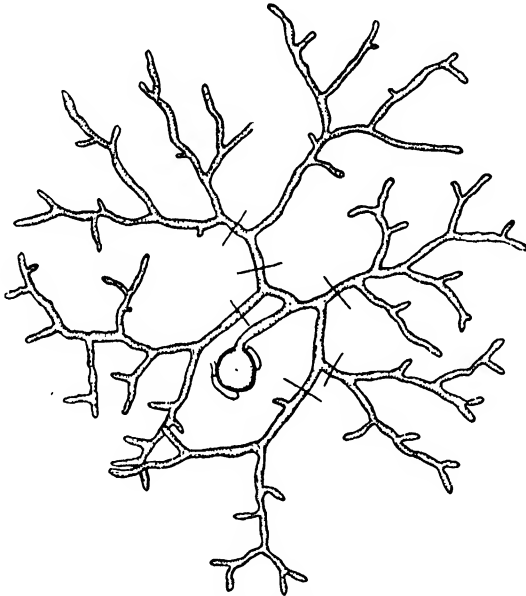
Several ripe eggs from cross 49 (A ♀ × B ♂ in corn-meal agar made on on January 14) were cut out and put in corn-grain juice February 22, and kept at room temperature, about 22° C. On February 28 the oil droplets were absorbed in most of the eggs. On March 1 fresh corn-grain juice was added. On March 2 the eggs were beginning to sprout, and on the following day the sprouts had developed into a slightly branched mycelium. Four sprouting eggs were teased out and put in fresh corn-grain juice, and were designated 49 f-i. By March 5, 10 a.m., the mycelium had grown into a loose web of threads about 0.8 cm. in diameter, but without the formation of any sporangia. The mycelia from eggs 49 f and 49 g were transferred to separate corn-meal agar plates. The mycelia from eggs 49 h and 49 i were each divided into two parts and each part was separately planted on a corn-meal agar plate, and after being cultured for a period of ten weeks, during which time the mycelia were transferred four times to fresh plates, numerous tests were made to determine the thallic character of the mycelia. Crosses were made as usual with strains A ♀ and 33 c ♂.

The mycelia from eggs 49 f and 49 g both gave reactions which indicated their mixed sexual nature, though in the case of 49 g the reaction with A ♀ was extremely weak, only two oogonia and no antheridia being seen in four crosses. Both parts of the mycelia from sprouting egg 49 h reacted sexually in all crosses with the stock male and female strains, thus showing that sexual differentiation was not completed in any parts of the mycelia tested.

In the crosses between the mycelia from egg 49 i and the stock male and female strains strikingly interesting results were obtained. In the

tests between the mycelia from one of the parts and the stock male and female strains, mixed reactions were obtained, but in the tests with the other parts a pure male reaction was obtained, thus indicating that sexual differentiation had taken place in this part.

In another sprouting egg, designated 210 e, the germinating hyphae were allowed to develop into a considerable mat of branched threads (Text-fig. 2). This mycelium was then divided into six parts (designated M 1-6) by cutting the threads about in the position shown by the cross-lines in Text-fig. 2, and each part was then transferred to a corn-meal agar plate. After being cultured on agar for about six weeks, during which time the



TEXT-FIG. 2. Sprouting egg 210 e.

fungus was transferred four times to fresh agar plates, cultures were made from all six of the mycelia and crossed in the usual way with the stock male and female strains. In these tests one of the parts of the mycelium gave a pure female reaction in all crosses, while all of the five other parts gave a mixed reaction.

These crosses brought to light several points of interest. The developing mycelium may become differentiated into several areas, certain of which may be apparently pure male, others pure female, and still other areas in which the two sexes are still undifferentiated, the segregation of the sex-determining substance taking place without the formation of any sporangia. In the three sets of crosses which produced a mixed reaction, the crosses between the stock male strain B and M 1, M 5, and M 6 pro-



duced many normal oogonia and antheridia, excepting one of the two crosses between B ♂ and M 6, in which no observable reaction took place. In the crosses between the stock female strain A and M 1, M 5, and M 6 only oogonial initials were formed, the oogonia usually proliferating considerably and becoming nearly empty of protoplasm, no antheridia being developed. It seems probable that in these latter crosses the mycelia of strains M 1, M 5, and M 6 contained both sexual natures, and that while the male nature was strong enough to stimulate the female to the formation of oogonial initials, it was not strong enough to develop antheridia to complete the formation of the sexual reproductive bodies. The reaction which B ♂ gave with M 1, M 2, M 5, and M 6 was quite remarkable and consistent with results formerly obtained in crosses between B ♀ and B ♂. In these crosses the antheridial strain was greatly stimulated, so that many of the oogonia were completely encircled with antheridia. Practically all the oogonia were furnished with antheridia, and a large majority of them were developing eggs. The very small number of the oogonia which were without antheridia had exhausted their contents by repeated proliferation.

The crosses between M 3 and B ♂, and M 4 and B ♂, showed no sexual reaction when first examined, while at the same time the crosses between A ♀ and M 3 and M 4 produced fairly strong reactions, though M 4 formed no antheridia. It was concluded, therefore, that M 3 and M 4 were antheridial strains. Upon a re-examination of the former crosses a week later, a few oogonia and antheridia were found in them, and therefore the mycelium in these strains, M 3 and M 4, had to be considered as of a mixed sexual nature. The crosses of the strain M 2, which was giving a female reaction, were also re-examined, but no oogonia or antheridia have been formed in the contrasts with strain A ♀. Strain M 2 seemed, therefore, to be female.

It seemed from the results obtained in the mycelial tests that sexual differentiation might take place in the mycelium, and that certain regions might be male and other regions female in nature, but that, due to the fact that as growth proceeds the threads of both sexes become intermixed, it usually happens in making tests that a mixed reaction results, since a good many threads are usually cut out with each agar square. In view of the results of Blakeslee (1) with *Mucor mucedo*, in which he found that the segregation of sex is completed at some time before the formation of sporangial spores, and that all the spores in a given germ sporangium were of the same strain, it seemed a matter of no small import to induce the early formation of sporangia in the germinating egg, and to test the sexual reactions of the spores. The germinating egg of *Dictyuchus*, as has already been seen above, sprouts into a germ-tube which soon branches into a mycelium. This mycelium, if kept in a liquid medium, usually

forms numerous sporangia. If the germ mycelium is transferred to practically pure water, the early formation of sporangia may be readily induced.

One of the germinating eggs, 33 d, from cross 33 sprouted into a germ-tube which, without branching, bore a single sporangium containing seven spores, three of which germinated as has been noted above (see Pl. XXXVIII, Fig. 9). These three spores were teased out with fine glass needles, and planted separately on corn-meal agar plates. The mycelia from these spores were then tested with the stock male and female strains. In four tests between cultures descended from each of the spores and the stock male strain B no reaction was obtained; while in the same number of tests with the female strain A oogonia and antheridia were formed in all of the crosses, thus proving the three spores to be antheridial in their sexual nature. Unfortunately, though several other efforts were made, no more eggs could be induced to germinate in this manner.

The mycelium of one of the five sprouting eggs, namely 49 j, from cross 49, tests of four of which have been discussed above, was transferred to sterile well-water on March 5. On the following day many sporangia had been formed. The early sporangia on the mycelium of a germinating egg are usually rather small, containing from eight to about fifty spores. One small sporangium containing about fifteen spores was separated out from the mycelium of 49 j and placed in a fresh drop of corn-grain juice. After about six hours the spores had sprouted, the germ-tubes growing out through the sporangial wall. The sporangium was then picked up with a glass needle with a knob on the end of it and transferred to a fresh agar plate, and there, under a low-power binocular, with very fine glass needles the sprouting spores were separated from each other, picked up on the end of a very fine glass needle with a small knob on the end, and placed on fresh agar plates (March 8, p.m.). After twenty-four hours eleven of the spores had developed a slightly branched mycelium. These eleven were designated j 1-11.

In order to ascertain the sex character of each of the eleven mycelia, several crosses were made between each of the eleven spores and strains A ♀ and B ♂ and 33 c ♂. In these crosses five of the eleven spores produced male mycelium, one female, four produced mixed mycelium, and one spore a neutral mycelium. Only two of the spore strains produced any reaction with B ♂, while of the six crosses between the spore strains and 33 c ♂ four produced oogonia and antheridia, thus indicating again the strong male nature of 33 c and the relatively weak male nature of B ♂.

Fifteen spores from another sporangium were isolated and tested as above. The strains descended from the sprouting spores were designated k 1-15. In these tests no pure males were found, while five spores produced mycelium which was female in nature, nine produced mycelium

which reacted with both the antheridial and oogonial strain, and one produced a mycelium neutral in its reactions.

Of the eleven spores tested in the first series, four produced antheridia when crossed with A ♀, and oogonia when crossed with 33 c ♂ or B ♂, and of the fifteen tested in the second series nine reacted both with the antheridial and oogonial strains. In most of the spores which produced a mixed mycelium the reaction was either considerably stronger male than female, or vice versa. As shown from the results with the fifteen spores in the second series, the four mixed mycelia gave a stronger reaction with the antheridial strain, 33 c, than with the oogonial strain A. In the crosses with the mixed mycelia, which produced a weak sexual reaction, the sexual organs were usually limited to a small area in the crosses, generally only a few threads bearing oogonia and a few bearing antheridia, the greater part of the threads, even where the two opposing strains were in contact, remaining sexually inactive. In spite of the fact that such mixed strains, when kept separated from strong oogonial or antheridial strains, have invariably failed to produce any sexual reproductive bodies, the production of such strains from a single spore capable of reacting with both the oogonial and antheridial strains would seem to indicate that such a mycelium was homothallic in nature.

It was a matter of interest to find out if the mycelia from these apparently homothallic spores continued to be homothallic after being cultured for a month or more, and if the mycelia from spores which gave a pure male or pure female reaction in the first tests would continue to do so. The mycelia from the spores k 1-15 were kept growing on corn-meal agar plates, and about six weeks after the first tests were made another series was made in the same way. In comparing the results in the two sets of crosses it is seen: that in the first series there were no pure males, while in the second two spore strains, k 9 and k 11, gave strong male reactions; that two of the five strains giving a pure female reaction in the first set gave a mixed reaction in the second set; that the strain which gave a neutral reaction in the first cross gave a mixed reaction in the second series; and that the number of mixed strains remained relatively the same.

The tests seem to indicate that the sex may be segregated in the early stages of egg germination, or that it may take place in the early formed sporangia, but that in these sporangia the segregation is only partial, as some of the spores give rise to mycelia which might be considered homothallic. Interesting in this connexion are the results obtained by Blakeslee (2) with *Phycomyces nitens*, in which he found that a segregation of sex may take place at the formation of spores in the germ sporangia, which, however, is only partial, as, in addition to (+) and (-) heterothallic spores, spores are formed which give rise to homothallic mycelia characterized by a production of contorted aerial outgrowths termed pseudophores and the

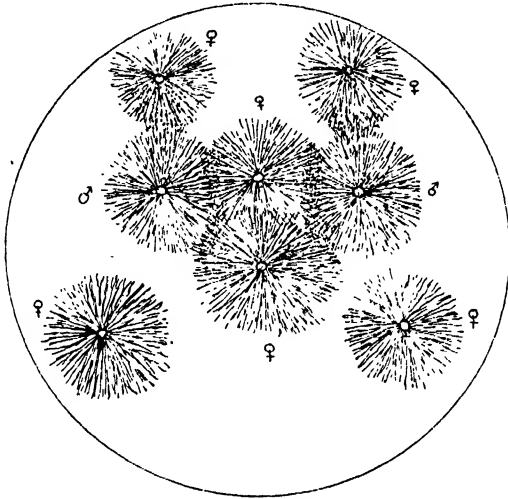
occasional formation of homothallic zygospores. He also reports that the sexual character in these homothallic mycelia is unstable, and in their sporangia a segregation again takes place and (+), (−), and homothallic spores are produced. The results obtained in the tests with germinating eggs in *Dictyuchus* furnish a striking parallel to those obtained by Blakeslee in *Phycomyces*.

#### MORPHOLOGY AND PHYSIOLOGY OF REPRODUCTION.

In species of the Saprolegniaceae in which sexual reproduction apparently takes place the sexual organs are of two very distinct morphological types, namely, the oogonia, which are usually comparatively large, and the antheridia, which are much smaller. In all species which have been carefully studied the oogonial initials appear first, the antheridial branches appearing later and growing to the oogonia. The oogonia, so far as observation has gone, do not seem to be attracted by the antheridia, but there seems to be a strong attraction on the part of the oogonia for the antheridia, as the latter quite often grow to the oogonia from a considerable distance. In species in which fertilization has been proved, as *Achlya de Baryana* by Trow (13) and *Saprolegnia monoica* by Claussen (8), antheridia are applied to the oogonial wall, antheridial cells are cut off, and part of the contents of the male gametangium is discharged into the developing egg.

In *Dictyuchus* the oogonial and antheridial strains are indistinguishable from each other when grown separately. The threads are all rather thick, only slightly branched, and bear numerous sporangia. If the two sexual strains are placed so that their threads are in contact, the two strains are considerably stimulated, finally resulting in the production of numerous sexual reproductive bodies. The stimulus often first manifests itself by more or less renewed growth, especially on the sides of the cultures where the threads are in contact. Usually, and especially in cultures which produce a strong reaction, numerous hyphae grow out from the substrata of the opposite cultures, these threads intermingling, often apparently anastomosing in places, and finally forming a dense mat of hyphae, those from the oogonial culture forming numerous oogonial initials, and those from the antheridial culture branching out into antheridial threads. Quite often, however, the oogonial and antheridial initials may arise from the primary threads. As is the case with monoecious species of the Saprolegniaceae, the oogonial initials are formed several hours before the antheridia come in contact with them. Oogonial initials may be formed on the side of the culture on threads which are not in contact with the threads from the antheridial culture. In some cases antheridial branches have been observed to grow a distance of two millimetres to such oogonia, in which case the oogonia formed eggs.

The question now arises as to the nature of the stimulus which causes the formation of the reproductive bodies. It seemed logical to assume that some substance was given off by the male plant which diffused through the water and stimulated the female in such a way that it formed oogonia. To test this hypothesis a female plant was put in the same Petri dish with a male, but placed so that the threads of the two plants were not in contact. After several days the two cultures were examined, but no observable change had taken place in either plant. This experiment has been repeated many times with the same result. In view of the possibility that the sexual



TEXT-FIG. 3. For explanation see text.

substance given off by one culture only might diffuse throughout the liquid and become too weak to cause any stimulation, a female strain was put into the dish with four young healthy antheridial cultures without changing the water in which the antheridial strains were growing, but still no oogonial initials were formed, nor were the antheridial strains in any recognizable way affected. Eight antheridial cultures were now used, but with the same result.

It might be that the sexual substance is given off only when the threads of the opposite strains are in contact. That such might be the case seemed to be suggested by the occurrence of oogonial initials out on the side of the female culture a considerable distance away from the region where the threads of the two opposite strains were in contact. To test the possibility of a stimulating substance being given off under such a condition, crosses were made between two female cultures and two male cultures as shown in the diagram, Text-fig. 3, and at the same time four oogonial cultures growing on bits of boiled corn grain were placed in the dish, two with their

threads barely crossed over those of the antheridial strain and two placed away from the antheridial strain a distance of about 4 mm. Upon examination several days later, many oogonia and antheridia were formed on the cultures in contact in the regions where the threads of the strains of opposite sex intermingled. Oogonia were also formed on the two oogonial cultures the threads of which were barely in contact with those of the antheridial strain. The threads of the antheridial strains had grown considerably, becoming so intimately associated with those of the latter two oogonial strains that even when the cultures were washed with a strong stream of water from a wash-bottle they were not separated. On the two oogonial cultures which were not placed in contact with the antheridial strains no oogonia were formed. This experiment has been repeated several times with the same results.

Other experiments have been made in which the two opposing strains were crossed as usual, but with a very thin collodion membrane separating the threads, so that the threads of the two strains could not come in contact and anastomose. In these experiments the female threads in the region covered by the collodion membrane became unhealthy in a large percentage of the cultures, due probably to a poor oxygen supply, but even in the cultures in which the covered threads remained healthy no sexual reaction occurred.

To obviate the possibility of a poor oxygen supply interfering with the reaction the following scheme was used: The male was placed on one side of a collodion membrane and the female on the opposite side, and then this was placed over a tall glass ring with the female on the under side of the membrane in a Petri dish filled with enough water to leave an air-space about 1 mm. deep in the ring. The sagging of the membrane made a nice bowl to contain water and thus prevent the male culture from drying, and at the same time pushed the hempsced on which the female was growing far enough into the water to keep that culture wet. No reaction was obtained, however, in any of these crosses. To obtain a still greater oxygen supply, bowl-shaped collodion membranes were used. With the male in about a centimetre of water on the inside of the bowl and the female opposite the male on the outer and under side, the bowl was glued to the top of the Petri dish chamber, which was filled with enough water to partially immerse the female culture. But in these tests no reaction was obtained.

Hard filter-paper was now used in place of the collodion membranes. Upon examining the cultures several days after they were made, many oogonia were found on the female culture. Both cultures stuck tenaciously to the filter-paper, indicating almost certainly that some of the threads had grown through the paper. Moreover, some of the oogonia had antheridia on them, and one oogonial initial was seen on the male side, but the thread

### *a Genus of the Water Moulds.*

bearing it was not connected with the male strain. Essentially the same results were obtained in several similar experiments.

It seems, therefore, from the data so far presented, that the stimulation initiating the sexual reaction passes through the anastomoses in the coenocytic mycelium.

An experiment was now carried out to determine if the potency of the active substance in the male which stimulated the female to the formation of oogonial initials was lost if the male was crushed and the juice pressed out. The expressed, filtered juice from the male. Eight young healthy cultures of the antheridial strain were crushed with a mortar and pestle and filtered with about 10 c.c. of water through a suction filter. A healthy young female was now put on a sterilized hollow-ground slide and the juice from the male poured over it. Upon examination several days later, the female was found to be quite healthy, but showed no sexual stimulation. This experiment was repeated several times with similar results. Other experiments were carried out in which the juice from the males was further diluted and no sexual stimulation was observed in the female. Antheridial strains were also put in juice expressed from oogonial strains, but no observable stimulation took place.

It seemed from these experiments that in order to stimulate the female to the formation of oogonial initials it was necessary to have a living healthy male actually in contact with the female culture.

To find out just how much of a male culture was required to stimulate the female to the formation of oogonial initials, the following tests were made: A single healthy thread was cut from a male culture and placed across the threads of a healthy female culture a couple of millimetres behind their tips. The male thread healed up at the cut end and remained apparently healthy, but produced no stimulation in the female. This experiment was repeated several times, using one male thread, two, three respectively, but all with the same results. Six threads were cut from the same male culture from which the threads used in the above experiments were cut, and placed across the threads of a female culture. When examined several days later, a considerable number of oogonia were formed on the female threads in the region where the male threads were in contact, and a few antheridial branches had grown out from the female threads, applying themselves to the oogonia.

Although no systematic attempt has been made to determine the effects of varying the external conditions on the formation of sexual reproductive bodies, yet it has been repeatedly noticed that alterations in temperature or food material produced variations in sexual activity. It has been found that crosses between certain strains produced oogonia more readily and in greater abundance when kept at a temperature about 26° C. while in crosses between most strains the optimum temperature for

formation of oogonia and antheridia seemed to be around 22° C. Two kinds of seeds have been used in the cultures more extensively than any others, namely, hempseed and corn grain. While the former has a particular advantage over the latter in that cultures on it are relatively free from bacteria, still cultures of opposite strains grown and crossed on the latter, if the bacteria can be kept out, produce oogonia and antheridia much more readily than cultures on the former. Moreover, considerable variation has been found to occur in sexual activity as the concentration of the food material was changed. Crosses of opposite strains on corn-meal agar made up according to our usual formula produced no sexual stimulus, while if the amount of the corn meal was doubled, as seen above, oogonia and antheridia were formed. It may be concluded here, as Blakeslee (1) did in the case of *Mucor mucedo*, &c., that variations in the external conditions have a secondary and variable effect in influencing sexual reproduction.

#### SUMMARY,

The strains of *Dictyuchus* as collected in nature show certain variations which, according to the general principles of classification in this group, would permit the making of several species, as has already been done.

In strains which were producing sexual fruits the antheridial and oogonial strains have been isolated and grown separately, under which conditions they invariably remain sexually sterile, but when the opposite strains are grown together so that their threads intermingle, oogonia and antheridia are formed in the region where the threads are in contact, thus proving the plant to be dioecious or heterothallic.

To date, six female strains, nine male, and eleven neutral strains have been collected.

The opposite strains, with a few exceptions, of the apparently different species may be intercrossed, in which crosses variations occur in the size of the oogonia and eggs and in the character of the antheridia which seem to invalidate any specific distinctions between the species *D. monosporus*, *D. Magnusii*, *D. carpophorus*, and *D. sterile*.

A heretofore unreported strain which forms oogonia and eggs without antheridia is described. In crosses between strains descended from the germinated parthenogenetic eggs and the male strain 33 c, the male strain, in a few cases, was stimulated to the formation of antheridia which applied themselves to the walls of the oogonia of the parthenogenetic strain. In 2 out of 98 crosses between the strains descended from the germinated parthenogenetic eggs and the female strain A oogonia were formed on the latter as well as on the parthenogenetic strain, and the parthenogenetic strain was stimulated to the formation of antheridia which applied them-



selves to the oogonia of strain A ♀; in which cases, as a rule, eggs were formed.

In numerous crosses between the male and female strains of *Dictyuchus* and other moulds sexual stimulation occurred only between the female strain A of *Dictyuchus* and *Thraustotheca primoachlya*, in which attempts the *Dictyuchus* formed oogonial initials and the *Thraustotheca* formed a superabundance of antheridial branches, the stimulation occurring only in the region where the threads of the two plants intermingled.

The eggs of *Dictyuchus*, after a rest period of a month to six weeks, become capable of germinating either into a short hypha which bears a single sporangium, or into a more or less branched mycelium.

Numerous eggs have been germinated and considerable experimentation has been done to find out when sexual segregation took place. It was found that partial sexual segregation may take place early in the egg germination, so that parts of the mycelium may be male, parts female, and parts may be mixed, or that segregation may take place at the formation of spores in the early formed sporangia, in which some of the spores may be male, part female, and part mixed.

Considerable experimentation has been carried on to determine the nature of the stimulus which causes the formation of the reproductive bodies. (It has been found that the reproductive bodies are formed only when the male and female strains are actually in contact.) A female culture placed in the same dish with as many as eight male cultures, but not in contact with any of them, was unaffected by the proximity. Cultures of the opposite sex crossed as usual, but with a collodion membrane between, gave no reaction; if, however, a hard filter-paper is placed between the two cultures, the threads grow into the paper, come in contact, and oogonia and antheridia are formed. Oogonial strains put in the juice expressed from antheridial cultures were apparently unaffected. If, however, as few as six threads are cut from the antheridial culture and placed across the threads of an oogonial culture, sexual stimulation is obtained in this region.

No extensive attempt has been made to determine the effects of external conditions on the formation of sexual reproductive bodies, yet it may be concluded that variations in the external conditions have a secondary and variable effect in influencing sexual reproduction.

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## EXPLANATION OF PLATES XXXV-XXXVIII.

Illustrating Dr. J. N. Couch's paper on Heterothallism in *Dictyuchus*.

## PLATE XXXV.

The upper photograph is of a cross between the female strain A and the male strain B, showing the thick region of oogonia and antheridia where the threads of the two strains intermingle. Stained with iron-alum haematoxylin. × 5.

The photomicrograph below shows an enlarged view of the region producing sexual organs in the above cross. The oogonia are the spherical or slightly sub-spherical objects. The antheridia, applied to the oogonia, are visible in some cases. None of the oogonia has yet formed eggs. The dark elongated bodies are sporangia. × 25.

## PLATE XXXVI.

Photomicrograph of the edge of a cross showing oogonial threads extending from the left bottom corner towards the top right corner, whereas the antheridial threads are coming to the oogonia from the upper left corner. Numerous sporangia are shown. The small circles are resting spores and spore cysts. × 100.

PLATE XXXVII.

Fig. 1. Sporangia borne in sympodia. Strain B.

Figs. 2, 3. Sporangia borne in rows. Strain I.

Fig. 4. Sporangium showing emerging spores. Strain C.

Fig. 5. Sporangium of the *Thraustotheca* type. Strain G.

Figs. 6, 7, 8. Habit sketch of oogonia, antheridia, and sporangia from the original culture of strain B.

Figs. 1, 2, 3,  $\times 90$ . Figs. 4, 5,  $\times 250$ . Figs. 6, 7,  $\times 670$ . Fig. 8,  $\times 250$ .

PLATE XXXVIII.

Fig. 1. Ripe egg of *Dictyuchus* showing eccentric oil drop partially buried in the larger mass of protoplasm.

Fig. 2. Egg beginning to germinate; the oil drop surrounded by protoplasm.

Fig. 3. Oil drop being resorbed, showing a frayed outline. Egg-wall being resorbed, also showing an irregular outline. Cytoplasm granular.

Figs. 4, 5, 6, 7. Progressive stages in sprouting eggs, showing diminution in the oil drop, thinning of the egg-wall, and swelling of the egg.

Fig. 8. Egg germinating into a hypha.

Fig. 9. Germ-tube which contained seven spores, three of which have germinated. The sexual nature of these three germinated spores was tested, as described in the text, and all three were found to be male in nature.

Fig. 10. Showing a part of the edge of the region where the threads of A ♀ and B ♂ were intermingling, the oogonial threads coming from the upper right edge of figure, the antheridial threads coming from the bottom of the plate. A few oogonia are not furnished with antheridia, but are forming eggs.

Figs. 1-9,  $\times 620$ . Fig. 10,  $\times 162$ .





Bath 557

COUCH — HETEROTHALLISM IN DICTYUCHUS.





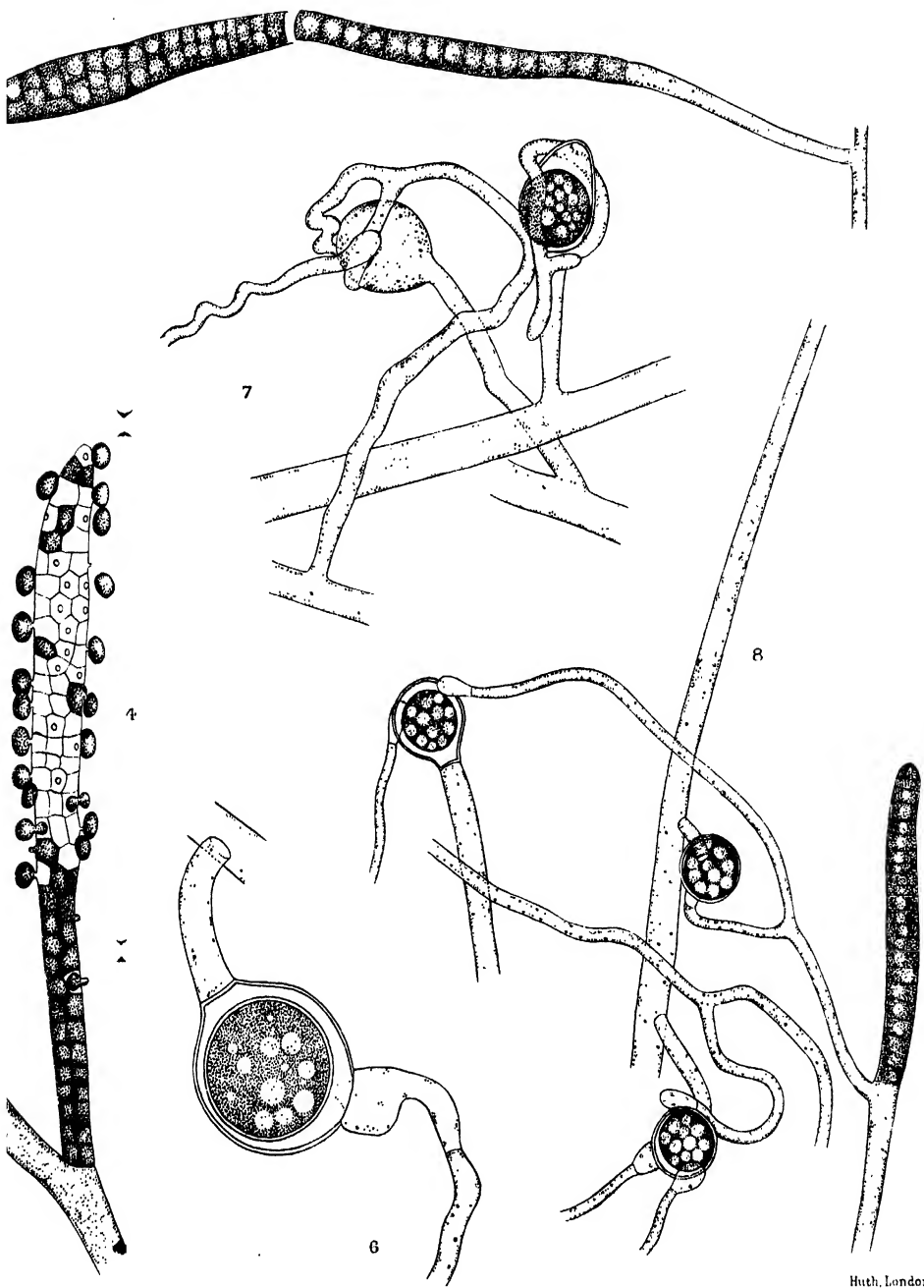
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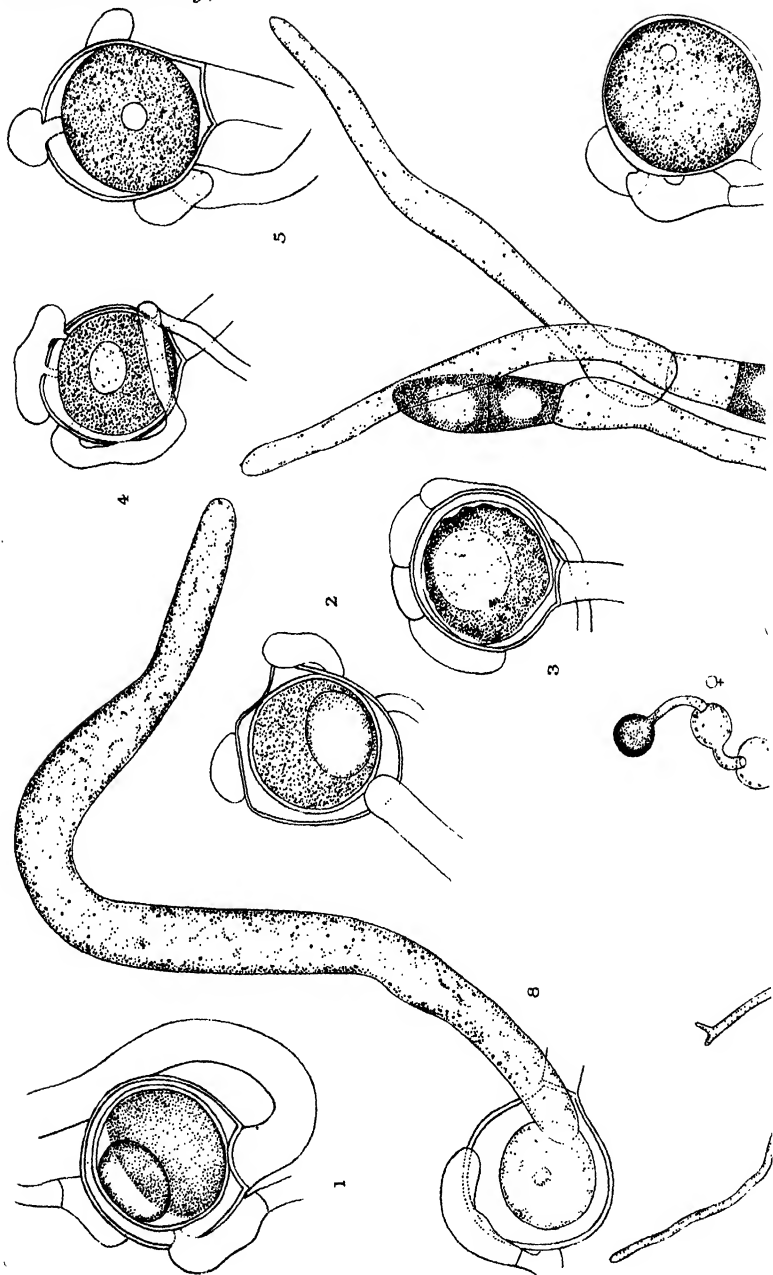
COUCH—HETEROTHALLISM IN *DICTYUCHUS*.



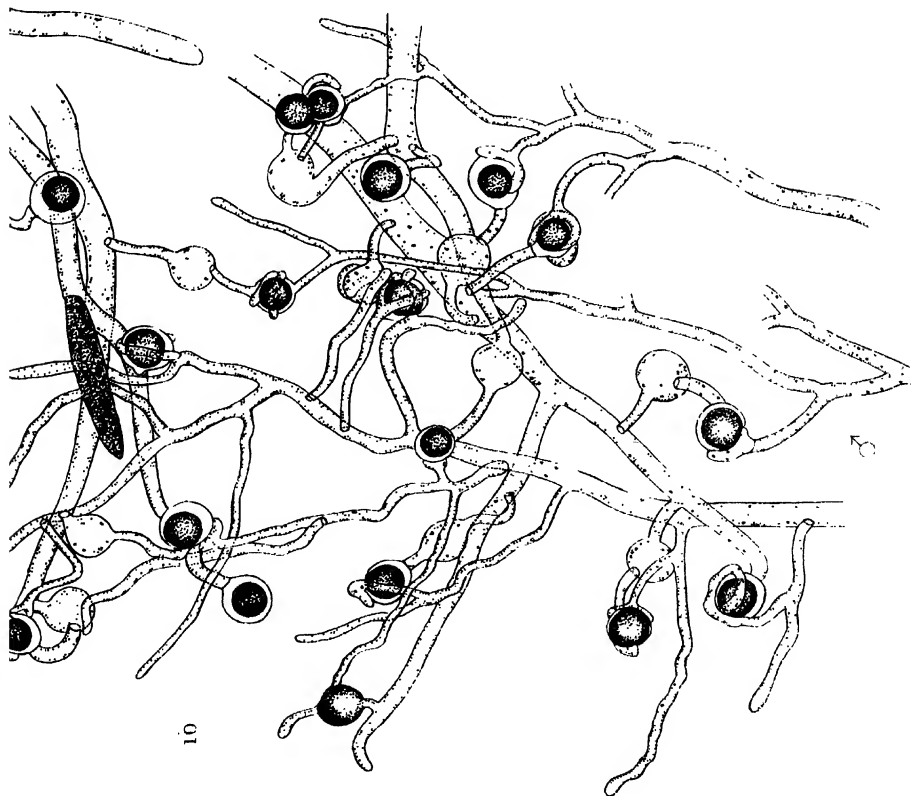
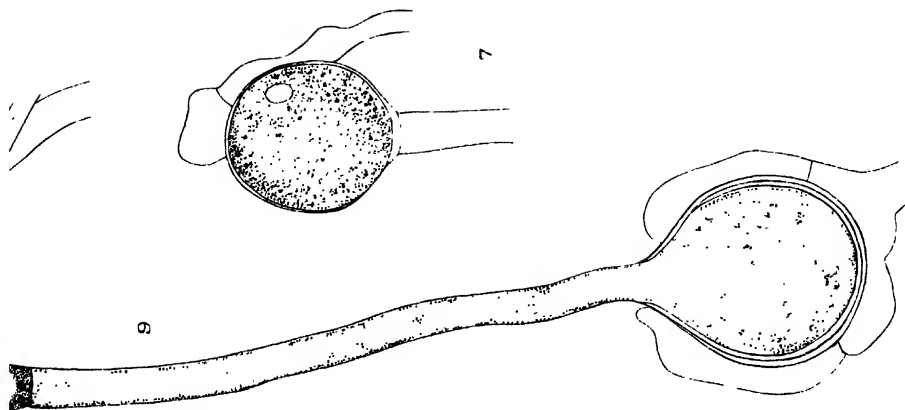
COUCH-HETEROTHALLISM IN *DICTYUCHUS*.







COUCH-HETEROTHALLISM IN DICTYUCHUS.





# Inoculation Experiments with *Nematospora gossypii*, Ashby and Nowell.<sup>1</sup>

BY

R. W. MARSH, B.A.

IN a previous investigation of a sample of diseased cotton from Nyasaland (2) it was found that the discoloration of the material was due to the presence of a yellow substance in the central canal of the hairs. The suggestion was put forward that this appearance, usually known as 'staining', was due to a pathological modification which had affected the contents of the developing hair cells. It was further suggested that the cause of the injury was an organism referred to as *Nematospora*, Species C, and now known as *Nematospora gossypii*. Although this fungus was abundantly present in the Nyasaland material, it was not in a viable condition, so it was not then possible to test this suggestion by experiment. In June 1925, however, Dr. W. Brown very kindly supplied me with cultures which made it possible to carry out the inoculations described below. The four organisms received were *Spermophthora gossypii*, *Nematospora gossypii*, *Nematospora coryli*—all originally from the West Indies—and a strain from Tanganyika Territory which Dr. Brown regarded as very near, if not identical with, Species C (now *Nematospora gossypii*).

It has been established by Nowell that in the West Indies these fungi are commonly associated with staining of the lint hairs of cotton, and that the agents bringing about inoculation of bolls in the field are usually the cotton-stainer bugs. In the present paper the action of the fungus is considered in the absence of any insect carrier. Apart from a mention by Nowell (3) of one boll successfully inoculated with *Nematospora coryli*, no records have been published of inoculations of cotton with pure cultures of the species of *Nematospora* and *Spermophthora*. It therefore appears desirable to put on record an account of some greenhouse inoculations which were carried out in the Experimental Grounds of Manchester University

<sup>1</sup> In a recent paper (1) by Ashby and Nowell, the boll-infecting fungi previously referred to as Species A, C, and D are named *Spermophthora gossypii*, Ashby and Nowell, *Nematospora gossypii*, Ashby and Nowell, and *Nematospora coryli*, Pegillon, respectively.

during 1925, even though the results may have only a limited application to the problems of infection in the field.

The organism employed for most of the inoculations was *Nematospora gossypii*, and the varieties of cotton<sup>1</sup> used were 'Mexican Big Boll', 'Delta-type Webber', 'Dixie Triumph', 'Cleveland', 'Sea Island', 'Ashmouni', 'Mit Afifi', and 'Sakel'. The plants were kept at a temperature which usually varied between 65° and 85° F. (extremes—60° and 110°). Under these conditions, untreated bolls developed in the usual manner and produced normal unstained lint. The bolls inoculated were first wiped with 90 per cent. alcohol, then pierced through the boll wall at the side with a fine needle carrying a small fragment of sporing mycelium from culture. Control punctures were made in a similar manner, using a sterile needle. In the majority of cases a single inoculation was made into a boll: occasionally the two opposite loculi of the same boll were inoculated, but in all the experiments only one puncture was made into each loculus. Some of the bolls employed were protected after inoculation by small paper bags, but this was not found to be of any advantage and was discontinued. A few inoculations were made in June and July; the remainder were carried out in the period between August 13 and October 10.

No infection resulted when a culture of *Nematospora gossypii* was placed on the outside of an unwounded boll. Detached bolls were set up in moist chambers in the laboratory and three or four fragments of sporing mycelium were placed at various points on the epidermis of each boll. After eight days there was no growth of the mycelium and no sign of infection. It was also found that in seven examples, where the mycelium was placed in the open flower on the living plant at the base of the corolla, no infection took place.

Puncture inoculations into developing bolls did not give successful infections unless the boll had reached an age of approximately two weeks. Of nineteen bolls, one week old or less, which were inoculated by placing sporing mycelium into the puncture made by a needle-prick, five were shed shortly after the treatment, and no infection took place in the remaining fourteen.

The inoculations made by puncturing older bolls may now be considered. As a rule the bolls were examined a fortnight after inoculation, and infection was recorded as successful only when a pure culture of *Nematospora gossypii* was re-isolated from a portion of the lint at least 1 cm. from the track of the puncture.

Table I sets out details of the inoculations into bolls more than one week old. The varieties used and the ages of the bolls inoculated are given, and the numbers of bolls inoculated are followed, in brackets, by the numbers

<sup>1</sup> For seed of the American varieties I have to thank the Coker Pedigreed Seed Company, of Hartsville, South Carolina, U.S.A.

of successful infections. One hundred and forty-nine inoculations were made with *N. gossypii*, and these were distributed over a total of seventy-six plants.

TABLE I.

		<i>Inoculations with N. gossypii.</i>							
Variety:—	Mexican Big Boll.	Webber.	Cleveland.	Dixie Triumph.	Sea Island.	Mil Affii.	Ash-mouni.	Sakel.	Totals.
Age of Boll.									
2 weeks	—	2 (0)	—	—	3 (0)	—	5 (1)	—	10 (1)
3 "	17 (10)	2 (0)	4 (3)	2 (2)	2 (1)	1 (1)	8 (1)	5 (0)	41 (18)
4 "	15 (1)	4 (0)	4 (1)	—	6 (3)	1 (0)	7 (2)	7 (2)	44 (9)
5 "	6 (2)	6 (4)	—	—	4 (1)	2 (0)	2 (1)	3 (1)	23 (9)
6 "	15 (4)	7 (1)	—	—	2 (0)	2 (1)	—	1 (0)	27 (6)
7 "	2 (0)	2 (0)	—	—	—	—	—	—	4 (0)
Totals	55 (17)	23 (5)	8 (4)	2 (2)	17 (5)	6 (2)	22 (5)	16 (3)	149 (43)

Of the unsuccessful inoculations, about 50 per cent. were contaminated by various organisms, *Cladosporium herbarum* being the most common. The rest of the punctured bolls remained sterile and produced lint which was either completely healthy or else showed a minute yellow speck marking the track of the puncture.

The total number of control punctures was 29. Ten of these were made on bolls less than 2 weeks old, and of this number 6 bolls were shed, 3 remained healthy, and 1 showed a stained speck of lint at the point of injury. The remaining 19 older bolls subjected to control punctures developed as follows: 11 remained healthy, in 2 the lint showed a yellow speck where punctured, 2 bolls were shed, and 4 became contaminated with *Cladosporium herbarum*.

The extent of the injury caused by the growth of a pure culture of *Nematospora gossypii* within the boll will now be described. Successful infection with this fungus gave rise to no indication on the exterior of the boll before dehiscence took place. On the inside of the boll wall there was commonly some formation of intumescence tissue at the point of inoculation, but this took place also around control punctures. The bounding membranes of the infected loculus, i.e. those of the dissepiments and of the outer wall, were often stained yellow, this colouring being due to a discoloration of their cell contents. If the lint had been infected when the boll was young, it showed a soaked appearance; this was not seen in bolls attacked when they had reached a later stage of development. The individual hairs in the infected region appeared more or less withered and remained irregularly twisted together. Staining of the hairs, as described in the previous paper

(2), was found in every case of successful infection. A patch of yellowed hairs commonly occurred around the point of inoculation and staining extended through the lint in all directions from this centre. A fortnight after inoculation stained hairs were present within a radius of about 1.5 cm. from the original puncture. No example was seen in which the attack had spread from one loculus to another. In the stained region the colour was most strongly marked at the base of the hairs. It was also noticeable that on the outer face of the mass of lint within the loculus staining was usually prominent in the groove which underlies the septum in the boll wall. The microscopic appearance of the stained hairs showed differences according to the age they had attained at the time when the injury took place. If the hairs were stained when the boll was 2–3 weeks old, the yellow pigment occurred in the central canal in discontinuous patches having indefinite outlines. Hairs attacked when the boll was 4–5 weeks old showed the yellow substance apparently filling long stretches of the central canal.

If an attacked boll was allowed to mature, it was found that, when dehiscence took place, the infected loculus often failed to open completely. The injured lint hairs remained in a tightly packed mass with a matted surface, instead of diverging in the characteristic loose manner of normal cotton.

The sporangial mycelium of *Nematospora gossypii* was found growing among the hairs in every direction, and was most evident at the margin of the stained region. The distribution of the fungus in the lint was approximately the same as that of the staining, but variations from a strict coincidence were frequent. This is not remarkable, for a nutritional disturbance brought about by the fungus at one portion of a hair might modify the cell contents for some distance beyond the attacked point.

No penetration of the hair walls by the fungus was seen and no spores or hyphae were found inside the hairs. The seeds were not found to be attacked in the course of this investigation.

The experiments described above establish the fact that *Nematospora gossypii*, when inoculated into developing lint in the boll, is capable of causing discoloration of the hairs (staining) due to modification of the protoplasmic contents of the living cells. The same effect was seen to be produced in a small number of examples in which successful infections were obtained with *Nematospora coryli*, *Spermophthora gossypii*, or the Tanganyika strain of *Nematospora gossypii*. A summary of the results of inoculating bolls more than one week old with these organisms is given in Table II, in which the figures already supplied for *N. gossypii* are reproduced for comparison.

It remains to be pointed out that staining of the lint can be caused by a number of agencies other than the organisms given in Table II. In fact, there appears to be no reliable criterion of infection by the species of *Nematospora* and *Spermophthora* apart from the recognition of the fungus in



the lint. Examples of staining due to other agencies have been frequently recorded by investigators in the field (3, 4) and a few such examples which were recorded during the course of these experiments may be briefly mentioned.

TABLE II.

*Summary of all Inoculations into Bolls more than One Week old.*

Inoculations with *Nematospora coryli*—7 (1 successful).

„ „ *Spermiophthora gossypii*—6 (4 successful).

„ „ Tanganyika strain of *Nematospora gossypii*—9 (5 successful).

„ „ *Nematospora gossypii*—149 (43 successful).

It was observed, as previously stated, that a needle puncture into a developing boll frequently caused the formation of a minute stained speck where the lint was pierced, although the contents of the loculus remained sterile and produced otherwise healthy lint. Also in the course of the present series of inoculation experiments extensive staining was often found in bolls in which the developing lint had been attacked by contaminating fungi or bacteria. *Cladosporium herbarum*, *Penicillium* sp., *Cephalosporium* sp., *Bacterium malvacearum*,<sup>1</sup> and unidentified species of bacteria were all found associated with staining in this way. Experiments on immature lint to obtain information on the subject of staining have not as yet provided any clear explanation of this phenomenon. A brief reference will be made to some of the facts observed. If a portion of seed-coat bearing immature hairs is kept in a hanging drop, it is found that the protoplasm of each hair breaks up into irregular masses in the central canal, but no staining takes place. Growth of *Nematospora gossypii* in the same drop does not appear to affect this result. It is necessary to state that in setting up these experiments some damage to the hairs was unavoidable, and it is probable that they were killed by the treatment received.

The effect was tried of inoculating living immature bolls with small quantities of phenol and silver nitrate respectively. The presence of these poisonous substances in contact with the developing lint did not cause staining. Slight staining took place in the lint of a detached unopened boll kept in chloroform vapour, but formaldehyde vapour similarly employed did not induce this effect.

In a number of other cases, where the contents of loculi from immature bolls were removed as a mass and placed in sterile tubes under moist conditions, staining was manifest after forty-eight hours. Usually, the lint was slightly damaged by removal from the bolls, and contaminating organisms were frequently evident to the naked eye after a further three days. The

<sup>1</sup> Lint attacked by *Bacterium malvacearum* is typically reduced to a black mass, but some of the hairs at the border of the blackened area have occasionally been observed to show the characteristics of staining as defined in this paper.

staining was strongly marked at the hair bases and was conspicuous in those areas where only a thin layer of lint overlies the seed. It did not appear that the staining could be produced merely by exposing immature lint to the air, since cutting away the wall of an unripe boll would not bring about staining as long as the exposed lint remained sterile.

The possible effect of *Nematospora gossypii* on the wall of the hair was investigated in the laboratory as follows: Tubes were set up containing a nitrogenous solution [ $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{NH}_4\text{NO}_3$ , and  $\text{KH}_2\text{PO}_4$ —each 0.5 gm. per litre] and mature cotton hairs taken from a freshly opened boll. After sterilization, these tubes were inoculated with the fungus and kept in an incubator at 24° C. for fifty days. Similar cultures, for shorter periods, were made in hanging drops. The fungus made scanty growth, but no shattering of the hairs took place in any of the cultures, as far as could be determined by microscopic examination. Under the same conditions the *Cephalosporium* stage of a *Fusarium* sp. (showing resemblances to *F. moniliforme*), which was known to be capable of attacking cellulose, brought about marked shattering in a week. In a similar manner, the relations of this organism to starch were investigated, and it was found that *Nematospora gossypii* displayed no power of attacking starch grains. Cultures of *Spermophthora gossypii* and *Nematospora coryli* acted in the same way as those of *N. gossypii*.

#### SUMMARY.

1. The position of *Nematospora gossypii* as a wound parasite of the developing cotton boll has been confirmed. It has been established that this organism is unable to attack unpunctured bolls or to cause injury to mature cotton hairs, and *N. gossypii* has shown no power of destroying cellulose under experimental conditions. The injury caused by the growth of a pure culture of this fungus in the developing lint is not a destruction of the existing cell walls, but appears to be an interference with the subsequent growth and maturing of the cotton hairs, this effect being marked by staining, arrested development, and, probably, premature death of the hairs.

2. Staining, a pathological modification of the cell contents, is produced only as a reaction of living hairs. It has been demonstrated that many agencies other than *Nematospora gossypii* are able to bring about this effect. Examples are given which show that staining results when immature lint is infected by any one of many different saprophytic fungi and bacteria, but the attempts to produce staining by purely physical means gave inconclusive results.

3. A small number of bolls were infected with pure cultures of *Spermophthora gossypii* and of *Nematospora coryli*, and the effect of these organisms on the developing lint was found to be similar in all respects to that produced by *N. gossypii*.

I wish to express my best thanks to Dr. W. Brown, of the Imperial College of Science, for his kindness in placing at my disposal the cultures which made this investigation possible. The work has been carried out under the general direction of Dr. Wilfrid Robinson, to whom I am greatly indebted for encouragement and helpful criticism.

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# The Distribution of Spores of Diverse Sex on the Hymenium of *Coprinus lagopus*.

BY

DOROTHY E. NEWTON, M.Sc. (McGILL).

With four Diagrams in the Text.

## I. INTRODUCTION.

THE observations of Mlle Bensaude, Kniep (6, 7), Miss Mounce (9), Vandendries (11, 12), Brunswik (1), Hanna (4), the writer (10), and others, made from 1918 onwards, have shown that, while certain species of Hymenomycetes are homothallic, other species are heterothallic. The experiments so far made indicate that in the Hymenomycetes there are far more heterothallic species than homothallic.

In a homothallic species of Hymenomycetes each spore gives rise to a mycelium which is at first haploid, but which, in the course of a few days, spontaneously becomes diploid. The haplophase, in which the nuclei are isolated from one another, is associated with simple septa, while the diplophase, in which the nuclei are in pairs and undergo conjugate division, is associated with the presence of clamp-connexions at the cross-walls. Sooner or later the diploid mycelium produces a diploid fruit-body, the pair of nuclei of opposite sex in each basidium fuse together, the fusion nucleus undergoes two successive divisions during which segregation of sex factors takes place, four haploid nuclei are thus produced, and these nuclei pass up into the four spores. The haploid spores thus brought into existence are ready to initiate the life-history once more.

In a heterothallic species each spore on the hymenium of an individual fruit-body is unisexual and, normally, the mycelium produced by each spore, so long as it is grown in isolation, continues to be of one and the same sex as the parent spore. As in a homothallic species, each spore and its branching germ-tube are haploid; but, unlike the homothallic species, a monosporous mycelium of a heterothallic species does not pass spontaneously from the haplophase to the diplophase, but continues in the diplophase until conjugation with another mycelium of opposite sex takes place.

Heterothallic Hymenomycetes are divisible into two groups: (1) species in which the spores on each fruit-body fall into two sexually opposite groups (haploid genotypes); and (2) species in which the spores on each fruit-body fall into four sexually different groups.

(1) In a heterothallic species with *two* sexually different kinds of spores on each fruit-body, e.g. *Coprinus radians* (11),<sup>1</sup> *C. Rostrupianus* (10), and *C. comatus* (1), normally the spores of one sex (after germination by means of their mycelia) react positively—forming pairs of nuclei, and giving rise to clamp-connexions—only with spores of the opposite sex. One Mendelian pair of factors may be considered as here involved. The diploid fusion nucleus in the basidium bears both factors and may be represented as (*Aa*). The haploid nucleus of each of the four spores which are produced on the basidium bears only one factor, so that sexually the two kinds of spores may be represented as (*A*) and (*a*). In a previous paper I have shown that in *C. Rostrupianus* all the basidia of any individual fruit-body are alike, in that they bear two spores of one sex and two of the other and opposite sex (10). Presumably this is true for other species resembling *C. Rostrupianus*, such as *C. radians* and *C. comatus*.

(2) In a heterothallic species with *four* sexually different kinds of spores on each fruit-body, e.g. *Aleurodiscus polygonius* (7), *Schizophyllum commune* (6), *Coprinus lagopus* (5), and *Hypholoma fasciculare* (3), normally one kind of spore (after germination by means of its mycelium) can react positively with only one of the other three kinds. Two Mendelian pairs of factors may be considered as here involved. The diploid fusion nucleus in the basidium bears both pairs of factors, and may be represented as (*AaBb*). The haploid nucleus of each of the four spores which are produced on the basidium bears only one of each pair of factors, so that, sexually, the four kinds of spores may be represented as (*AB*), (*ab*), (*Ab*), and (*aB*).

In species which on any individual fruit-body have four sexually different kinds of spores (*AB*), (*ab*), (*Ab*), (*aB*), it is of interest to determine the relative numbers and positions of the four kinds of spores on the hymenium which covers the gills. Towards the solution of this problem certain data have already been obtained by Kniep (7), Funke (3), Hanna (5), and myself (2, 10).

Kniep, in 1922, observed that in *Aleurodiscus polygonius*, under certain abnormal moisture conditions, the four spores of each basidium are shot

<sup>1</sup> Vandendries working with *Coprinus radians* (12) and the writer (10) working with *C. Rostrupianus* have observed that, while in these species all the monosporous mycelia are at first unisexual (haploid) and as such can be mated in the usual way, when these unisexual mycelia are kept in culture for several weeks or months a large percentage of them spontaneously become bisexual (diploid), as is indicated by the spontaneous development of clamp-connexions. This phenomenon, which is of considerable theoretical interest, requires further experimental elucidation. Species in which the monosporous mycelia are at first unisexual and subsequently bisexual have been called by Vandendries *hetero-homothallic*.

away together in a single mass. Taking advantage of this fact, he procured the spore-masses of thirty-five basidia. Each spore-mass was deposited in sterilized agar in a flask, and the four spores were then separated from one another by shaking the flask. After the spores had germinated, the four mycelia were isolated and then mated with one another in all possible ways. Kniep found that each of his thirty-five basidia had produced two kinds of spores, two of one sex and two of another and opposite sex. The four spores of some of the basidia contained the sex factors  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$ , while the four spores of the other basidia contained the sex factors  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ . Kniep therefore concluded that the reduction process in the basidium takes place in the *first* division of the fusion nucleus and not in the second. Subsequently, he informed Professor A. H. R. Buller *in litt.* (March, 1925) that in further experiments with *Aleurodiscus polygonius* he had discovered a few basidia which had borne all four kinds of spores  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$ , so that he could no longer maintain that in this species the reduction process always takes place in the first nuclear division.

In October, 1924, Funke (3) announced that with the help of a micro-manipulator he had succeeded in removing the four spores of a few individual basidia of *Hypholoma fasciculare*, *H. capnoides*, and *Collybia velutipes*, in sowing the spores separately in culture media, and in analysing each set of four spores for their sex reactions. In *Hypholoma fasciculare* two basidia were of the type  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$ , and four of the type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$ . In *Collybia velutipes* one basidium was of the type  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$ , and three others of the type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$ . In *Hypholoma capnoides* similar results were obtained, but the number of basidia used was not stated. In none of these species, doubtless owing to the small numbers of basidia analysed, did Funke obtain a third type of basidium:  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ . He concluded from his results that, where a basidium had produced only two kinds of spores, the reduction process had taken place in the first division of the fusion nucleus and that, when a basidium had produced all four kinds of spores, the reduction process had taken place in the second division.

In November, 1924, Buller (2), without being aware of the publication of Funke's paper, gave a summary of some experiments on sex which had been made in his laboratory. He described the cover-glass-contact method for isolating the four spores of a single basidium; and he announced that Hanna had found two types of basidia on the gills of *Coprinus lagopus*,  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$ , and  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$ , and that Newton had found that all the basidia on the hymenium of any fruit-body of *Coprinus Rostrupianus* were alike in that each of them bears two spores of one sex and two of another and opposite sex.

Hanna (4), in April, 1925, gave a detailed account of his experiments

on *Coprinus lagopus*. Of thirteen basidia analysed, seven bore two spores of one sex and two spores of another and opposite sex, while six bore spores of all four kinds ( $AB$ ), ( $ab$ ), ( $Ab$ ), ( $aB$ ). He stated that in the basidia with two spores of one sex and two of another and opposite sex there were in all probability two types present, namely ( $AB$ ), ( $AB$ ), ( $ab$ ), ( $ab$ ), and ( $Ab$ ), ( $Ab$ ), ( $aB$ ), ( $aB$ ), but he did not carry out any mating experiments to test his supposition (5).

In January, 1926, I stated (10) that I had analysed about twenty basidia of *Coprinus Rostrupianus* for the sexual reactions of their spores, and had found that every one of these basidia had borne two spores of one sex and two of another and opposite sex. There were no exceptions to this rule. Therefore I concluded that of the spores on any fruit-body of *C. Rostrupianus*, 50 per cent. are of one sex and 50 per cent. of another and opposite sex.

In the present paper, an attempt is made to extend our knowledge of the distribution of spores of diverse sex on the hymenium of *Coprinus lagopus*. Hanna, as we have seen, found that on a single fruit-body some of the basidia bore all four kinds of spores ( $AB$ ), ( $ab$ ), ( $Ab$ ), ( $aB$ ), and other basidia spores of two kinds only, but he did not demonstrate that of the latter some basidia were of the type ( $AB$ ), ( $AB$ ), ( $ab$ ), ( $ab$ ), and others of the type ( $Ab$ ), ( $Ab$ ), ( $aB$ ), ( $aB$ ), although he regarded this as probable. Analyses of the sexual reactions of the four spores of numerous individual basidia have been made for the following purposes: (1) to determine whether or not the three types of basidia ( $AB$ ), ( $ab$ ), ( $Ab$ ), ( $aB$ ), and ( $AB$ ), ( $AB$ ), ( $ab$ ), ( $ab$ ), and ( $Ab$ ), ( $Ab$ ), ( $aB$ ), ( $aB$ ), occur on the hymenium of any individual fruit-body, as supposed by Hanna; (2) to obtain data as to the relative numbers of the three types of basidia on any individual fruit-body; and (3) to determine in respect to sex the relative positions of the four spores on individual basidia, in the hope of throwing some light on the reduction process.

## II. METHODS.

Some fruit-bodies of *Coprinus lagopus* came up spontaneously on fresh horse-dung balls contained in a large covered dish in the laboratory. A spore-deposit produced by the pileus of a single fruit-body was collected on a sterilized glass slide, and then many of the spores were sown together on sterilized horse-dung in a quart jar plugged with cotton-wool. After about fourteen days the mycelium began to yield fruit-bodies, and fruiting continued for several weeks. This culture, which was the product of a single sexual strain,<sup>1</sup> afforded material for most of the experiments, and

<sup>1</sup> Hanna (5) has shown that *Coprinus lagopus* is made up of many sexual strains, any two of which are perfectly fertile *inter se*, i.e. any monosporous mycelium of one strain will give a positive sexual reaction with any monosporous mycelium of the other strain.



was always employed where intercrossings between the spores of two or more basidia from the same or from diverse fruit-bodies were undertaken.

The four spores of single basidia were removed from the hymenium by the cover-glass-contact method (2, 5, 10), and then the individual spores of each tetrad were sown separately in dung-agar by the dry-needle method (4). A thin layer of dung-agar was poured into a Petri dish, and after it had set four circles, each about 1 cm. wide, were drawn in a row with a wax pencil on the under side of the dish, and numbered in succession 1, 2, 3, and 4. In order to keep a record of the relative positions of the four spores of a tetrad derived from a single basidium, the spores were removed by the needle in counter-clockwise succession: the first spore removed was set in the agar above the circle No. 1, the second spore above the circle No. 2, and so forth for spores No. 3 and No. 4.

In using the technique just described there are certain difficulties involved. (1) The four spores of a tetrad attached to the cover-glass, while separate from one another, are only a few microns apart, and to remove them one by one with the needle in counter-clockwise succession without allowing any one to touch its fellows requires steadiness of hand and some practice. If, after spores No. 1 and No. 2 had been successfully removed from a tetrad, spores Nos. 3 and 4 became stuck together accidentally, the original relative positions of the spores Nos. 3 and 4 were lost, and the whole tetrad had to be discarded so far as its value for determining the relative positions of the spores of diverse sex was concerned; but, after spores Nos. 3 and 4 had been separated by manipulations with the needle and had been sown separately, the tetrad could still be used for determining the nature of the sexes of the four spores, and thus for adding to the statistics concerning the relative numbers of the three types of basidia which occur on the hymenium. (2) It not infrequently happens that of the four spores of a tetrad which have been successfully isolated in counter-clockwise succession, and have been sown in their proper sequence above the four rings in the Petri dish, only one or two germinate. One is then obliged to discard the tetrad for all purposes. When three spores germinate, the tetrad can be further investigated advantageously; for, when the sexes of three of the four spores in any tetrad are known, one can readily infer to which of the three kinds of tetrads, namely (*AB*), (*ab*), (*Ab*), (*aB*); (*AB*), (*AB*), (*ab*), (*ab*); and (*Ab*), (*Ab*), (*aB*), (*aB*), the tetrad under investigation belongs.

When all four spores of a single tetrad, or three spores of the four germinated, the individual monosporous mycelia, when about two days old, were transferred to separate Petri dishes. The culture medium was always dung-agar, which was prepared in the usual manner (10). The method of pairing the monosporous mycelia on agar plates was the same as that described by Hanna (5).

The criteria of sex employed were those now generally in use in experimental work on hymenomycetous fungi (9, 10). The chief criterion was the presence or absence of clamp-connexions after two monosporous mycelia had been paired on dung-agar in a Petri dish. Normally, a monosporous mycelium, when grown for an indefinite period in isolation, never develops any clamp-connexions. When two monosporous mycelia have been paired on a plate, if, after union, they develop clamp-connexions, they are considered as being of opposite sex; whereas if, after union, they fail to produce clamp-connexions, they are considered as having one or both sex factors in common. Sexual union may be effected by the following pairings,  $(AB) \times (ab)$ ,  $(Ab) \times (aB)$ , but not by the following pairings,  $(AB) \times (AB)$ ,  $(ab) \times (ab)$ ,  $(Ab) \times (Ab)$ ,  $(aB) \times (aB)$ ,  $(AB) \times (aB)$ ,  $(AB) \times (Ab)$ ,  $(Ab) \times (ab)$ ,  $(ab) \times (aB)$ .

While the clamp-connexion criterion was employed in every experiment, another criterion, namely, the presence or absence of oidia, was also very generally used, although in a subsidiary manner. Haploid mycelia not only do not produce clamp-connexions, but they develop oidia freely, and the oidia give to the surface of the mycelium a more or less floury appearance. On the other hand, diploid mycelia, while producing clamp-connexions, never develop oidia, and the surface of such mycelia is never floury to the naked eye. When two monosporous mycelia have been paired, therefore, the cessation of oidia-production as the mycelia grow radially outwards indicates that the two mycelia are of opposite sex, whereas the continued production of oidia indicates that the two mycelia possess either one or both sex factors in common.

### III. DISCUSSION OF THE THREE THEORETICAL TYPES OF BASIDIA.

Granted that a fruit-body of *Coprinus lagopus* bears spores of four sexually different kinds and that each spore bears two sex factors, one belonging to one pair of factors and the other to another pair of factors, the spores can be represented by the symbols  $(AB)$ ,  $(ab)$ ,  $(Ab)$ , and  $(aB)$ . Then there are three types of basidia of which the existence seems probable, and they may be represented by the sex factors of their spores:  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$ ;  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$ ; and  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ .

When the four spores of a basidium of the type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$  are crossed in all possible ways between themselves, we obtain a result like that shown in Table I. Each monosporous mycelium gives clamp-connexions with *one* only of the three other mycelia. The only pairs of mycelia which give clamp-connexions are those which have no sex factors in common. In all the tables a (+) sign indicates that, after pairing, clamp-connexions appeared in the culture, and a (−) sign that they did not.

When the four spores of a basidium of the type  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$  are crossed in all possible ways between themselves, we obtain a result like that shown in Table II. Each monosporous mycelium gives clamp-connexions with *two* of the other three mycelia. Here, again, the only pairs of mycelia which give clamp-connexions are those which have no sex factors in common.

TABLE I.

	AB	ab	Ab	aB
AB	—	+	—	—
ab	+	—	—	—
Ab	—	—	—	+
aB	—	—	+	—

*Bas. I.*

TABLE I. All possible pairings of the four monosporous mycelia from a basidium of the type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$ .

TABLE II.

	AB	AB	ab	ab
AB	—	—	+	+
AB	—	—	+	+
ab	+	+	—	—
ab	+	+	—	—

*Bas. II.*

TABLE II. All possible pairings of the four monosporous mycelia from a basidium of the type  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$ .

TABLE III.

	Ab	Ab	aB	aB
Ab	—	—	+	+
Ab	—	—	+	+
aB	+	+	—	—
aB	+	+	—	—

*Bas. III.*

TABLE III. All possible pairings of the four monosporous mycelia from a basidium of the type  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ .

TABLE IV.

	AB	AB	ab	ab
Ab	—	—	—	—
Ab	—	—	—	—
aB	—	—	—	—
aB	—	—	—	—

*Bas. II × Bas. III.*

TABLE IV. All possible pairings of the four monosporous mycelia from a basidium of the type  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$  with the four monosporous mycelia from a basidium of the type  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ .

When the four spores of a basidium of the type  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$  are crossed in all possible ways between themselves, we obtain a result like that shown in Table III. Each monosporous mycelium gives clamp-connexions with *two* of the other three mycelia. Here, as in the other types of basidia, the only pairs of mycelia which give clamp-connexions are those which have no sex factors in common.

If now the four spores of a basidium of the type  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$  are paired in all possible ways with the four spores of a basidium of the type  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ , the result to be expected should be that shown in Table IV, for in every pair of mycelia there will be one sex factor in common, and this will prevent the formation of conjugate pairs of nuclei and of clamp-connexions. Thus Table IV shows negative results only.

After pairing the four spores of one basidium with the four spores of another basidium: if (1) we obtain half-positive and half-negative results like those shown in Tables II and III, we may be quite sure that the two basidia crossed are of the same type, and either  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$ , or  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ , although we cannot tell which; whereas, if (2) we obtain entirely negative results like those shown in Table IV, we may be

TABLE V.

		Bas. II.				Bas. III.			
		AB	AB	ab	ab	Ab	Ab	aB	aB
Bas. I.	AB	—	—	+	+	—	—	—	—
	ab	+	+	—	—	—	—	—	—
	Ab	—	—	—	—	—	—	+	+
	aB	—	—	—	—	+	+	—	—

All possible pairings of the four monosporous mycelia from each of the two types of basidia  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$  and  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$  with the four monosporous mycelia of a basidium of the type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$ .

equally sure that the two basidia are of different types, one of them being of the type  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$  and the other of the type  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ . If, arbitrarily, we give one set of symbols to one of the two groups of four monosporous mycelia, then we must give the other set of symbols to the other group.

If the four spores of a basidium of the type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$  are paired with the four spores of a basidium of the type  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$ , and also with the four spores of a basidium of the type  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ , theoretically we obtain results like those shown in Table V. Let us call the three basidia shown in the table Basidium I, Basidium II, and Basidium III, so that their numbers correspond to the three types just mentioned and in the same respective order. Then we perceive, by reference to Table V, that the four spores of Basidium II do not react with the spores  $(Ab)$  and  $(aB)$  of Basidium I, nor the four spores of Basidium III with the spores  $(AB)$  and  $(ab)$  of Basidium I, owing to the fact that in all these pairings one sex factor would be common to both mycelia. The positive

reactions between Basidia I and II are limited to pairings between (*AB*) and (*ab*) spores which have no sex factors in common; and, similarly, the positive reactions between Basidia I and III are limited to pairings between (*Ab*) and (*aB*) spores.

#### IV. AN EXPERIMENTAL PROOF OF THE EXISTENCE OF THE THREE TYPES OF BASIDIA.

(1) In the first series of experiments thirty basidia were investigated. Fifteen gave results like that shown in Table I, which indicates that they had produced spores of four different sexes, i. e. were quadrisexual, while fifteen gave half-positive and, half-negative results like those shown in Tables II and III, which indicates that they had produced two spores of one sex and two spores of another and opposite sex, i. e. were bisexual. Without further investigation, however, as explained in the preceding section, it could not be known whether the fifteen bisexual basidia were all of the type shown in Table II, or were all of the type shown in Table III, or were some of one type and some of the other.

Of the fifteen basidia which bore spores of two kinds in pairs, eleven were selected for further analysis. In five of these all four spores had germinated, and in the remaining six, three spores only. Thus there were in hand a total of thirty-eight monosporous mycelia. These were then paired in all possible ways with a view to determining which were of the type shown in Table II and which of the type shown in Table III. The total number of pairings was 741, and the results were set out in a large table of which a representative section, embodying examples of all the kinds of reaction obtained, is shown in Table VI. In this table, the roman numerals attached to the basidia indicate the order in which the basidia were investigated by themselves before being paired with other basidia, while the arabic numerals indicate spores in the order in which they were removed from each tetrad, so that both kinds of numbers were chosen arbitrarily. The Mendelian symbols are those we seem compelled to adopt to explain the experimental results.

In Table VI it will be seen that the spores of three basidia (excepting one spore of Basidium VI which did not germinate) have been paired in all possible ways. The monosporous mycelia from Basidia VI and XIV gave no clamp-connexions with those of Basidium XIII. The monosporous mycelia from Basidia IX, XII, XIX, and XXVI behaved towards those from Basidium XIII exactly like those of Basidia VI and XIV. Hence we may conclude that Basidia VI, IX, XII, XIV, XIX, and XXVI are all of one and the same sexual type. The monosporous mycelia of Basidia XVIII, XXII, XXIV, and XXVII exactly resembled those of Basidium XIII in that they failed to react with the mycelia of Basidia VI, IX, XII, XIV,

XIX, and XXVI. Hence it is apparent that Basidia XIII, XVIII, XXII, XXIV, and XXVII belong to the same sexual type, but to one differing from that of Basidium VI and its like. If now to the spores of Basidia XIII, XVIII, XXII, XXIV, and XXVII we assign the symbols  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$ , then to the spores of Basidia VI, IX, XII, XIV, XIX, and XXVI we must assign the symbols  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ . Thus of the eleven

TABLE VI.

		<i>Bas. VI</i>			<i>Bas. XIII</i>				<i>Bas. XIV</i>			
		<i>Ab</i>		<i>aB</i>	<i>AB</i>		<i>ab</i>		<i>Ab</i>		<i>aB</i>	
		1	3	2	1	2	3	4	1	4	2	3
<i>Bas. VI</i>	<i>Ab</i>	1	—	—	+	—	—	—	—	—	+	+
		3	—	—	+	—	—	—	—	—	+	+
	<i>aB</i>	2	+	+	—	—	—	—	+	+	—	—
<i>Bas. XIII</i>	<i>AB</i>	1	—	—	—	—	+	+	—	—	—	—
		2	—	—	—	—	+	+	—	—	—	—
	<i>ab</i>	3	—	—	—	+	+	—	—	—	—	—
		4	—	—	—	+	+	—	—	—	—	—
<i>Bas. XIV</i>	<i>Ab</i>	1	—	—	+	—	—	—	—	—	+	+
		4	—	—	+	—	—	—	—	—	+	+
	<i>aB</i>	2	+	+	—	—	—	—	+	+	—	—
		3	+	+	—	—	—	—	+	+	—	—

*Coprinus lagopus*. All possible pairings of eleven monosporous mycelia from three different basidia, with a Mendelian interpretation of the results.

basidia which bore spores of two kinds in pairs, selected for further analysis, five were of the type shown in Table II and six of the type shown in Table III.

The above series of experiments clearly shows that, as supposed on theoretical grounds by Hanna, on the hymenium of any fruit-body of *Coprinus lagopus* there are three types of basidia the spores of which can be represented sexually as:  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$ ;  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$ ; and  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ .

(2) In a second series of experiments the following basidia were procured: Basidium XL with four kinds of spores and Basidia XXXIX, XLIII,

XLV, XLVI, XLVII, and XLVIII, all of which, when analysed singly, were found to have produced only two kinds of spores in pairs. Of Basidia XXXIX, XL, XLIII, and XLV all the four spores had germinated, and of Basidia XLVI, XLVII, and XLVIII only three of the four. Thus there were in hand twenty-five monosporous mycelia. These were paired, not *inter se* as in the first series of experiments, but with the four mycelia of Basidium XXXV, which was of the four-sex type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$ . The experimental results are embodied in Table VII. The roman and arabic numbers have the same significance as that already given for Table VI, and, in arranging the table, for clearness and making comparisons the like monosporous mycelia for each of the basidia have been placed together. The symbols  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$  for the monosporous mycelia of Basidium XXXV were chosen arbitrarily. The symbols for the

TABLE VII.

Type I				Type II								Type III																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			
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*Coprinus lagopus*. All possible pairings of twenty-five monosporous mycelia from seven different basidia with four monosporous mycelia from a basidium that produced spores of four different sexes. The results show that the seven basidia include representatives of all the three possible types.

monosporous mycelia of the other seven basidia are those which it was necessary to choose in order to explain the experimental results.

An examination of Table VII shows: (1) that Basidium XL gave with Basidium XXXV results like those shown in Table I, i. e. what we should expect theoretically when the four mycelia from each of two basidia both of the type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$  are paired together in all possible ways; (2) that Basidia XXXIX, XLIII, and XLVIII gave with Basidium XXXV results like those shown in the left half of Table V, i. e. what we should expect theoretically when the four mycelia from a basidium of the type  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$  are paired with the four mycelia from a basidium of the type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$ ; and (3) that Basidia XLV, XLVI, and XLVII gave with Basidium XXXV results like those shown in the right half of Table V, i. e. what we should expect theoretically when the four mycelia from a basidium of the type  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$  are paired with the four mycelia from a basidium of the type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$ . Hence

we may conclude from this second series of experiments that all the three types of basidia, which were postulated theoretically, were actually present together in one and the same hymenium.

#### V. THE RELATIVE NUMBERS OF THE THREE TYPES OF BASIDIA.

In the course of several months a total of forty-seven basidia were analysed for the sexual constitution of their spores. Four monosporous mycelia were obtained from the four spores of some of the basidia, but only three from others, owing to deficient germination. As there are only three types of basidia on the hymenium of *Coprinus lagopus* and as these types are so different from one another, when the sexual constitution of three of the four spores has been ascertained by experiment, one can say with certainty to which of the three types the basidium belongs. Hence, for our present inquiry, basidia of which only three spores germinated are just as useful as those of which all four spores germinated.

From the spores of the forty-seven basidia a total of one hundred and sixty-six monosporous mycelia was obtained. The mycelia from the spores of each basidium were first paired among themselves in all possible ways, as in Tables I, II, and III, so as to determine which of the basidia had borne spores of all four sexes ( $AB$ ), ( $ab$ ), ( $Ab$ ), ( $aB$ ), and which had borne spores of only two sexes in pairs; and then the mycelia from the basidia which had borne spores of only two sexes in pairs were paired in the manner illustrated in Tables VI and VII so as to determine which of them were of the type ( $AB$ ), ( $AB$ ), ( $ab$ ), ( $ab$ ), and which of the type ( $Ab$ ), ( $Ab$ ), ( $aB$ ), ( $aB$ ). The statistical results of the investigation may be summarized as follows:

1. Twenty-five basidia belonged to the ( $AB$ ), ( $ab$ ), ( $Ab$ ), ( $aB$ ), or four-sex type.
2. Twenty-two basidia belonged to the two-sex types, of which seventeen were further investigated in order to assign them to their respective types. Of these seventeen basidia:
  - (a) Nine belonged to the ( $AB$ ), ( $AB$ ), ( $ab$ ), ( $ab$ ) type, and
  - (b) Eight belonged to the ( $Ab$ ), ( $Ab$ ), ( $aB$ ), ( $aB$ ) type.

The data just recorded indicate: (1) that the number of basidia of the four-sex type ( $AB$ ), ( $ab$ ), ( $Ab$ ), ( $aB$ ) occurring upon the hymenium of any fruit-body is approximately equal to the number of basidia of the two two-sex types taken together; and (2) that the two two-sex types of basidia, namely, ( $AB$ ), ( $AB$ ), ( $ab$ ), ( $ab$ ), and ( $Ab$ ), ( $Ab$ ), ( $aB$ ), ( $aB$ ), are present on the hymenium in approximately equal numbers.

Expressed in percentages, the data given above indicate that of the three



types of basidia situated on the hymenium of any fruit-body of *Coprinus lagopus* :

- 50 per cent. are of the type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$ ,
- 25 per cent. are of the type  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$ , and
- 25 per cent. are of the type  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ .

These statistics will be further discussed in the next section.

## VI. THE RELATIVE POSITIONS, IN RESPECT TO SEX, OF THE FOUR SPORES OF INDIVIDUAL BASIDIA.

### A. *Experimental Results.*

The relative positions, in respect to sex, of the four spores of each of thirty-one basidia were determined by the methods already described in Section II. The results were as follows :

1. Twenty basidia bore spores of all four sexes. Of these there were :
  - (a) Eight with the  $(aB)$  spore in a diagonal position to the  $(AB)$  spore,
  - (b) Four with the  $(Ab)$  spore in a diagonal position to the  $(AB)$  spore,
  - (c) Eight with the  $(ab)$  spore in a diagonal position to the  $(AB)$  spore.
2. Eleven basidia bore spores of two sexes in pairs. Of these in the  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$  basidia there were .
  - (d) Four with an  $(aB)$  spore in a diagonal position to an  $(Ab)$  spore,
  - (e) None with an  $(Ab)$  spore in a diagonal position to an  $(Ab)$  spore, while in the  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$  basidia there were :
  - (f) Six with an  $(ab)$  spore in a diagonal position to an  $(AB)$  spore, .
  - (g) One with an  $(AB)$  spore in a diagonal position to an  $(AB)$  spore.

In the accompanying diagram (see p. 904) are shown the seven possible arrangements of the spores of diverse sex on the basidia. The inner circle in each spore represents a nucleus, and the symbols within a pair of sex factors. The number in the centre of each basidium body gives the actual number of basidia found with the arrangement of spores represented.

### B. *Theoretical Discussion.*

Theoretically, where there is only *one pair* of sex factors, the segregation of these factors<sup>1</sup> in the fusion nuclei of the basidia of any hymenium

<sup>1</sup> Reduction of the total number of chromosomes and the segregation of sex factors during the two divisions of the fusion nucleus are not identical phenomena. In this paper the phenomenon of segregation is of first interest, and hence the use of the term reduction will be avoided.

may take place in three possible ways: (1) in all the basidia in the first division of the nucleus; (2) in all the basidia in the second division; and (3) in some basidia in the first division and in other basidia in the second division.

In *Coprinus lagopus*, in any individual fruit-body, to explain the occurrence of four sexually different kinds of spores, it has been found

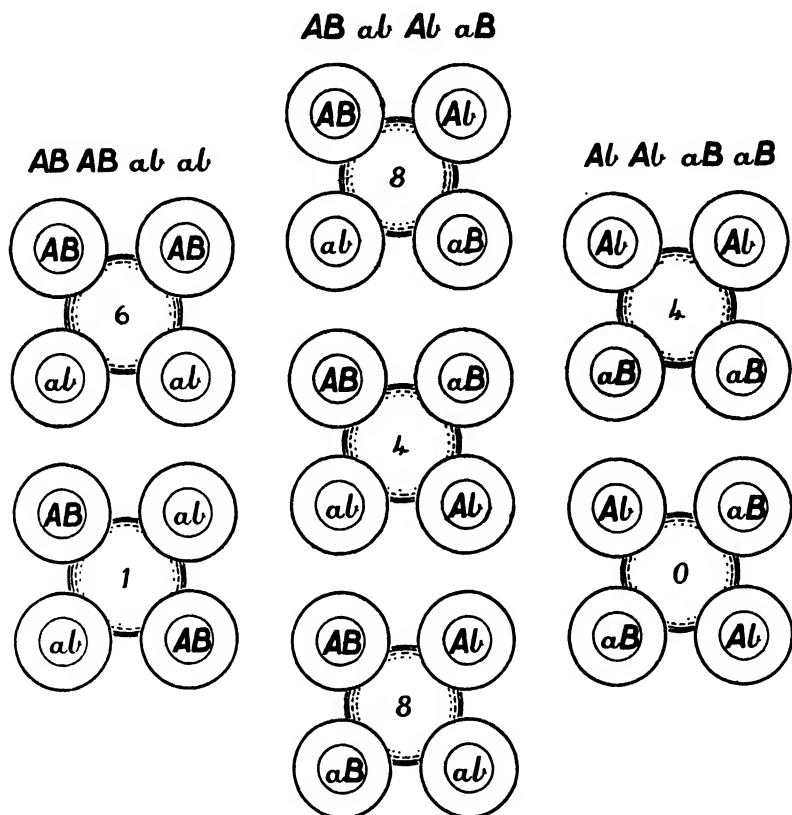


DIAGRAM 1. Showing the arrangement of the spores of diverse sex on thirty-one basidia of *Coprinus lagopus*.

necessary to assume that *two pairs of Mendelian factors are involved*, (*Aa*) and (*Bb*), and that each spore bears only one factor of each pair. The four kinds of spores possible on this assumption are: (*AB*), (*ab*), (*Ab*), and (*aB*).

In seeking for an explanation of the experimental data, it seems necessary to assume that each spore of *Coprinus lagopus* bears its two sex factors *not in the same chromosome but in two chromosomes*, one chromosome containing one factor and another chromosome the other factor.

Theoretically, where there are two pairs of sex factors, as in *Coprinus*

*lagopus*, the segregation of the sex factors in the fusion nuclei of the basidia of any hymenium may take place in seven possible ways. Let

*A* = segregation of both pairs of factors in the *first* division of the fusion nucleus,

*B* = segregation of both pairs of factors in the *second* division, and

*C* = segregation of one pair of factors in the *first* division, and of the other pair of factors in the *second* division.

Then the seven possible ways in which segregation may take place are as follows :

- (1) in all the basidia as in *A* ;
- (2) in all the basidia as in *B* ;
- (3) in all the basidia as in *C* ;
- (4) in some basidia as in *A*, in other basidia as in *B* ;
- (5) in some basidia as in *A*, in other basidia as in *C* ;
- (6) in some basidia as in *B*, in other basidia as in *C* ; and
- (7) in some basidia as in *A*, in other basidia as in *B*, and in yet other basidia as in *C*.

These various modes of segregation will now be discussed *seriatim* with a view to discovering which of them best fits the experimental facts.

(1) *In all the basidia segregation of both pairs of factors in the first division of the fusion nucleus.* Assuming that each of the two nuclei of opposite sex about to fuse in a young basidium contains two chromosomes bearing sex factors, and that segregation takes place in the manner just indicated, Scheme I represents what should happen during the process. Here, as in Schemes II and III which follow, the circles represent nuclei, the quadrilaterals chromosomes, and the nuclei are supposed to be viewed from the top of the young basidium, the observer looking down on the hymenium. The outlines of the spores and of the basidium wall are not shown. The five lines joining any two nuclei indicate that the two nuclei are daughter nuclei of a single parent nucleus.

At the top of Scheme I is shown a pair of nuclei of opposite sex, in a basidium, about to fuse, each having two chromosomes bearing sex factors. Two other nuclei, (*Ab*) and (*aB*), might have been substituted for the (*AB*) and (*ab*) nuclei actually here represented. The nuclei fuse (first large circle) with a pairing of (*A*) with (*a*) and of (*B*) with (*b*). Then in this fusion nucleus the chromosomes split (second large circle). Now segregation takes place in the first division of the fusion nucleus. There are two possibilities: the (*AA*) chromosomes may go with the (*BB*) chromosomes, and the (*aa*) with the (*bb*), as shown on the left, or the (*AA*) chromosomes may go with the (*bb*) chromosomes, and the (*aa*) with the (*BB*), as shown on the right. Finally, after the second nuclear division we get the arrangement of the nuclei shown at the base of the scheme.

It is known that the four nuclei resulting from the second division of the fusion nucleus make their way to the top of the basidium body and become attached there by their centrosomes. Above each centrosome the cell-wall grows upwards to form a sterigma and then a spore. The four nuclei then creep up their respective sterigmata, and so become introduced into the four spores (8). We shall assume that the four nuclei produced by divisions like those shown at the base of Scheme I are developed in a plane situated transversely to the basidium axis, as is usual in the higher Hymenomycetes, and that they fix themselves to the top of the basidium in the same relative order as that in which they come into existence in the first place. On this assumption, which remains to be verified cytologically, the relative positions of the four spores of any basidium, from the sexual point of view, are identical with the relative positions which were occupied by the four nuclei in the basidium body. The four circles of each group at the base of Scheme I, and of Schemes II and III which follow, therefore represent in respect to sex the relative positions of the four nuclei produced from the fusion nucleus in (1) the basidium body where the nuclei were formed, and in (2) the four spores into which they moved.

Does Scheme I, in which the segregation of both pairs of sex factors takes place in all the basidia in the first division of the fusion nucleus, fit the experimental facts? Evidently not: it does not provide for the occurrence of any basidia of the four-sex type, yet of the forty-seven basidia analysed, twenty-five were four-sex and twenty-two two-sex. Scheme I, therefore, does not account for one-half of the experimental facts, and for this reason alone it must be discarded. Scheme I yields two kinds of basidia only:  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$ , and  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ ; but, as we shall see, these two-sex basidia, theoretically, can come into existence by segregation taking place in the second nuclear division.

(2) *In all the basidia segregation of both pairs of factors in the second division of the fusion nucleus.* Assuming that each of the two nuclei of opposite sex about to fuse in a young basidium contains two chromosomes bearing sex factors, and that segregation takes place in the manner just indicated, Scheme II represents what should happen during the process.

Scheme II begins like Scheme I. At the top is shown a pair of nuclei of opposite sex, in a basidium, about to fuse, each having two chromosomes bearing sex factors. Two other nuclei,  $(Ab)$  and  $(aB)$ , might have been substituted for the  $(AB)$  and  $(ab)$  nuclei actually here represented. The nuclei fuse (first large circle) with a pairing of  $(A)$  with  $(a)$  and of  $(B)$  with  $(b)$ . Then in this fusion nucleus the chromosomes split (second large circle). There is no segregation in the first division of the fusion nucleus, so that each of the daughter nuclei, as shown, must contain all four sex factors  $(A)$ ,  $(a)$ ,  $(B)$ , and  $(b)$ . As a result of the second division of the fusion nucleus, as shown, there are six possible positions for the four nuclei. As in Scheme I,

the five lines joining any two nuclei indicate that the two nuclei are daughter nuclei of a single parent nucleus. The numbers placed in the centre of each group of four nuclei indicate how many of the thirty-

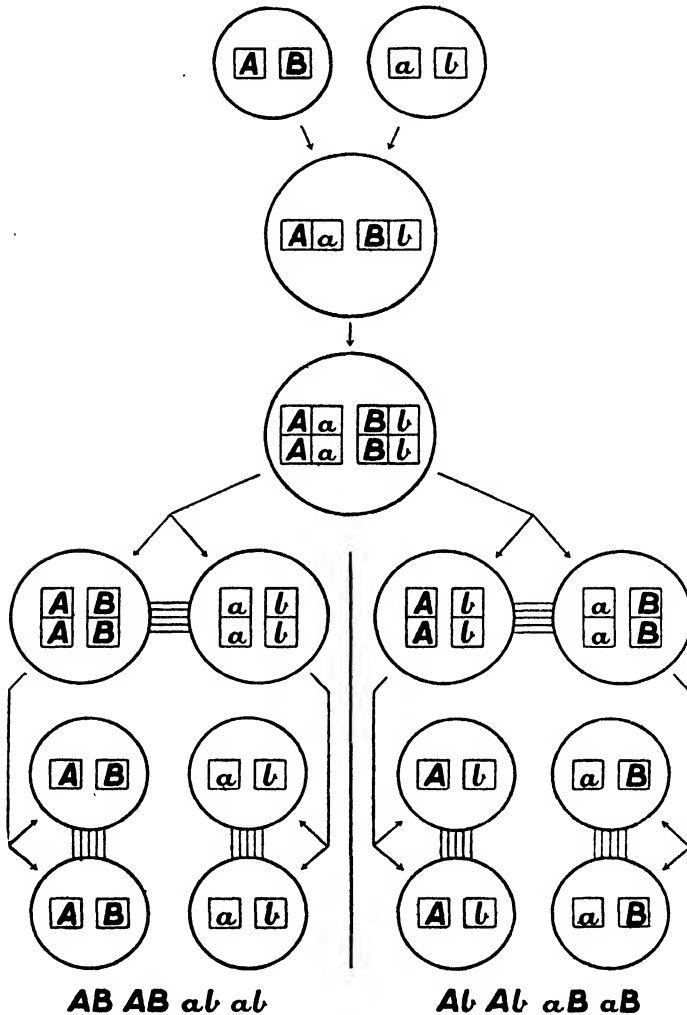


DIAGRAM 2. Scheme I, in which all the basidia show segregation of both pairs of sex factors in the first division of the fusion nucleus.

one basidia investigated had their spores arranged in the positions represented.

A survey of Scheme II shows that it provides: (1) for the occurrence of all three types of basidia ( $AB$ ), ( $Ab$ ), ( $ab$ ); ( $AB$ ), ( $Ab$ ), ( $aB$ ), ( $ab$ ); and ( $AB$ ), ( $ab$ ), ( $Ab$ ), ( $aB$ ); and (2) for the arrangement of the spores

of each type of basidium in two different ways, as may be seen by comparing the spores in diagonal positions.

It is not difficult to perceive that: (1) a basidium of the four-sex type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$  should be formed just as often as a two-sex basidium of the type  $(AB)$ ,  $(ab)$ ,  $(AB)$ ,  $(ab)$ ; and that (2) a basidium of the four-sex type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$  should be formed just as often as a two-sex basidium of the type  $(Ab)$ ,  $(aB)$ ,  $(Ab)$ ,  $(aB)$ . In other words, according to the law of chance, other things being equal, Scheme II with segregation wholly in the second division should give us 50 per cent. of basidia of the four-sex type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$ , 25 per cent. of two-sex basidia of the type  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$ , and 25 per cent. of two-sex basidia of the type  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ .

Another theoretical deduction from Scheme II is that the two arrangements of the spores of each type of basidium should occur with equal frequency. Thus in the  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$  type we should have  $(AB)$  and  $(ab)$  in diagonal positions just as often as  $(AB)$  and  $(AB)$ ; in the  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$  type we should have  $(AB)$  and  $(aB)$  in the diagonal position just as often as  $(AB)$  and  $(Ab)$ ; and in the  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$  type we should have  $(Ab)$  and  $(aB)$  in the diagonal position just as often as  $(Ab)$  and  $(Ab)$ .

Does Scheme II, in which the segregation of both pairs of sex factors takes place in all the basidia in the second division of the fusion nucleus, fit the experimental facts? The answer is: To a large extent, but by no means completely.

Firstly, Scheme II, unlike Scheme I, provides for all the three types of basidia. Secondly, it provides for the three types occurring in the right numerical ratio; for, as we have seen, it was found by experiment with forty-seven basidia that there are 50 per cent. of basidia of the  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$  type, 25 per cent. of the  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$  type, and 25 per cent. of the  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$  type.

With regard to the theoretical and actual positions of spores of diverse sex of the thirty-one basidia investigated, our Scheme II to a large extent is unsatisfactory. The actual number of basidia found with spores in the positions shown in each of the six sets of four in the lower half of the scheme is indicated by a central number. In the centre column we have 8 and 4 instead of the theoretical 6 and 6. The agreement between experiment and theory here is perhaps as great as might be expected with such small numbers; but, in the first column, we have 6 and 1 instead of 3 in one arrangement and 4 in the other; while in the third column we have 4 and 0 instead of 2 and 2. Theory and actuality, therefore, are very divergent in the first and third columns. The scheme does not provide at all for basidia of the four-sex type in which  $(AB)$  and  $(ab)$  are in diagonal positions, as may be seen by looking down the central column; yet 8 such

basidia were found in the 31 investigated. For this reason, in particular, Scheme II does not sufficiently explain the experimental data.

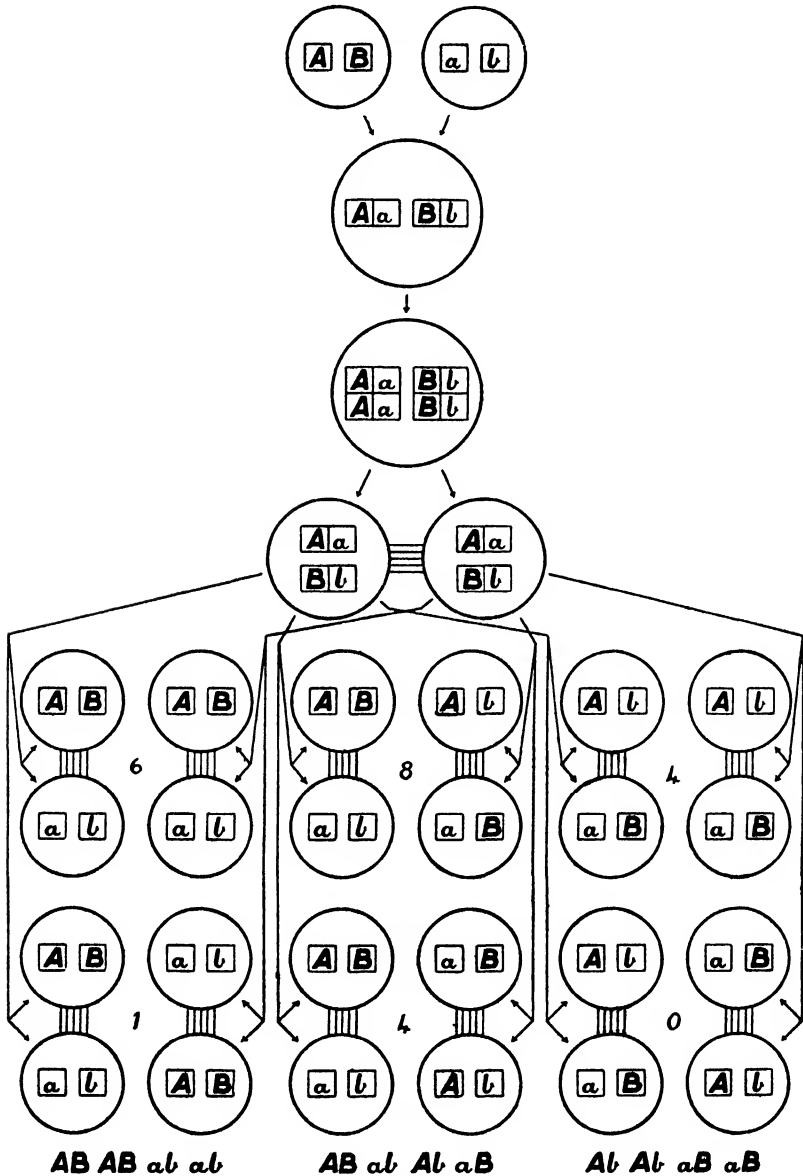


DIAGRAM 3. Scheme II, in which all the basidia show segregation of both pairs of sex factors in the second division of the fusion nucleus.

(3) In all the basidia segregation of one pair of factors in the first division of the fusion nucleus and segregation of the other pair in the second

*division.* Assuming this type of segregation to take place, it might be represented by a scheme like Scheme III, but with the outer ( $AB$ ), ( $AB$ ), ( $ab$ ), ( $ab$ ), and ( $Ab$ ), ( $Ab$ ), ( $aB$ ), ( $aB$ ) columns removed. It would yield exclusively four-sex basidia of the type ( $AB$ ), ( $ab$ ), ( $Ab$ ), ( $aB$ ).

It was found by experiment with forty-seven basidia that 50 per cent. of the basidia on the hymenium of a fruit-body belong to the two-sex types collectively, and 50 per cent. to the four-sex type. It is evident that the kind of segregation here under discussion, since it fails to yield any two-sex basidia, does not account for one-half of the experimental facts, and must therefore be discarded.

(4) *In some basidia segregation of both pairs of factors in the first division of the fusion nucleus, and in other basidia segregation of one pair of factors in the first division, and of the other pair in the second division.* Assuming that each of the two nuclei of opposite sex about to fuse in a young basidium contains two chromosomes bearing sex factors, and that segregation takes place in the manner just indicated, the following Scheme III represents what should happen during the process.

Scheme III begins with the two alternative possibilities for the formation of the fusion nucleus. Right and left we have a pair of nuclei of opposite sex, in a basidium, about to fuse, each nucleus having two chromosomes bearing sex factors. The nuclei of each pair fuse (first large circle) in such a manner that ( $A$ ) joins ( $a$ ) and ( $B$ ) joins ( $b$ ). Then in this fusion nucleus the chromosomes split (second large circle). In the first division of the fusion nucleus there are, as shown, four possibilities. In the two outer columns the two daughter nuclei show complete segregation, so that each nucleus contains only two kinds of sex factors, and not all four kinds. In the two central columns, the two daughter-nuclei show partial segregation, and this is of such a nature that each nucleus contains three of the four kinds of sex factors; and the process of segregation thus begun in the first division of the fusion nucleus is completed only in the second division. As a result of the second division of the fusion nucleus, as shown, there is only one possible position for the four nuclei in each of the two outer columns, but two possible positions for the four nuclei in each of the two central columns. However, since the top set of four in the left-hand central column is really nothing more than the looking-glass picture of the top set of four in the right-hand central column, the positions of the nuclei in these two sets may be considered as the same. As in Schemes I and II, the five lines joining any two nuclei indicate that the two nuclei are daughter nuclei of a single parent nucleus. The numbers placed in the centre of each group indicate how many of the thirty-one basidia investigated had their spores arranged in the positions represented. Since the top fours of the two central columns have essentially the same arrangement, and there were eight basidia with this arrangement, the number eight has been placed between them.



A survey of Scheme III shows that it provides: (1) like Scheme II, for the occurrence of all three types of basidia ( $AB$ ), ( $Ab$ ), ( $ab$ ); ( $AB$ ), ( $Ab$ ), ( $aB$ ), ( $ab$ ); and ( $AB$ ), ( $Ab$ ), ( $Ab$ ), ( $aB$ ); (2) for the arrangement of the ( $AB$ ), ( $AB$ ), ( $ab$ ), ( $ab$ ), and ( $Ab$ ), ( $Ab$ ), ( $aB$ ), ( $aB$ ) basidial types in one way only, and not in two ways as in Scheme II; and (3) for the arrange-

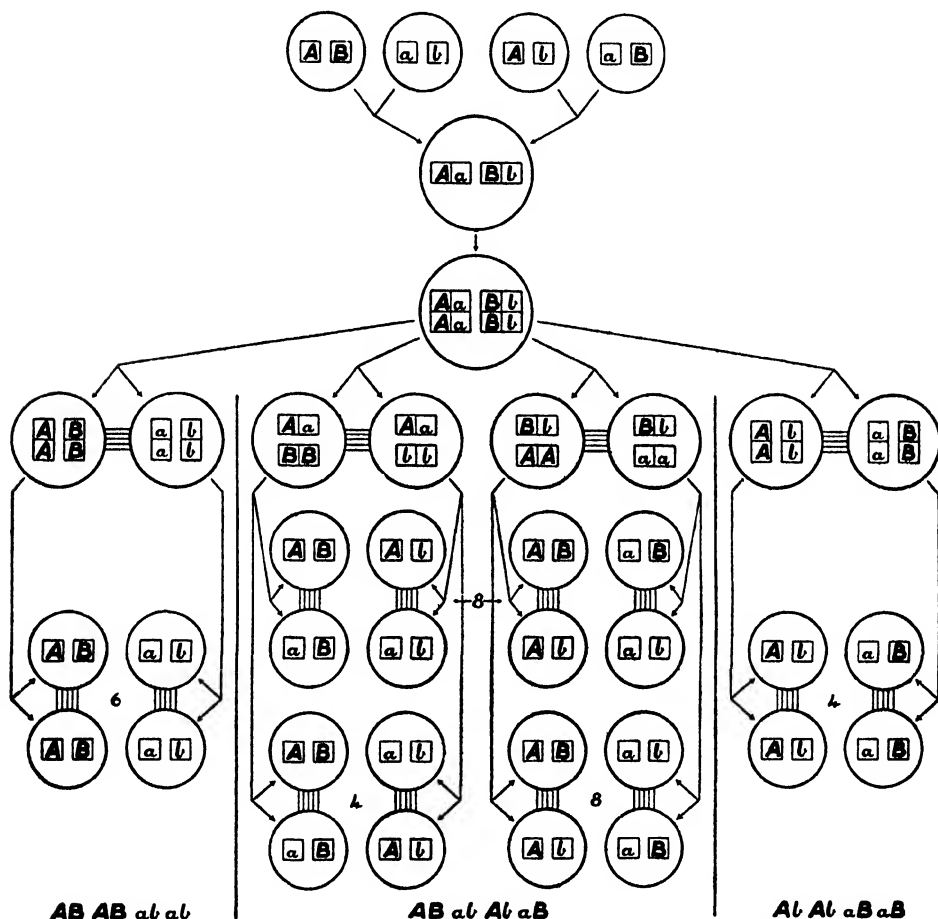


DIAGRAM 4. Scheme III, in which some basidia show segregation of both pairs of sex factors in the first division of the fusion nucleus, and other basidia show segregation of one pair of factors in the first division and of the other pair in the second division.

ment of the ( $AB$ ), ( $ab$ ), ( $Ab$ ), ( $aB$ ) basidial type in three ways, the diagonal to ( $AB$ ) being ( $ab$ ) or ( $Ab$ ) or ( $aB$ ), whereas in Scheme II the only possible diagonals to ( $AB$ ) are ( $Ab$ ) and ( $aB$ ).

Here, as in Scheme II, it is not difficult to perceive that: (1) a basidium of the four-sex type ( $AB$ ), ( $ab$ ), ( $Ab$ ), ( $aB$ ) should be formed just as often as a two-sex basidium of the type ( $AB$ ), ( $ab$ ), ( $AB$ ), ( $ab$ ); and that a basidium

of the four-sex type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$  should be formed just as often as a two-sex basidium of the type  $(Ab)$ ,  $(aB)$ ,  $(Ab)$ ,  $(aB)$ . In other words, according to the law of chance, other things being equal, Scheme III should give us 50 per cent. of basidia of the four-sex type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$ , 25 per cent. of two-sex basidia of the type  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$ , and 25 per cent. of two-sex basidia of the type  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ .

In Scheme III, unlike Scheme II, there is only one and not two possible arrangements of the spores on each of the two types of two-sex basidia  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$  and  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$ .

In Scheme III the two upper sets of four in the middle columns, where  $(ab)$  is diagonal to  $(AB)$ , although different in origin, are, as already pointed out, simply mirror pictures of one another. Theoretically, therefore, in the four-sex basidia, we should expect the arrangement where  $(ab)$  is diagonal to  $(AB)$  to occur twice as often as the arrangement where  $(Ab)$  is diagonal to  $(AB)$ , and also twice as often as the arrangement in which  $(aB)$  is diagonal to  $(AB)$ .

Does Scheme III, in which in some basidia the segregation of both pairs of sex factors takes place in the first division of the fusion nucleus, while in other basidia segregation of one pair takes place in the first division and of the other pair in the second division fit the experimental facts? The answer is: Very well indeed except for a single basidium.

Firstly, Scheme III, like Scheme II, provides for all the three sexual types of basidia. Secondly, it provides for the three types occurring in the right numerical ratio; for, as we have seen, the data obtained from an investigation of forty-seven basidia indicate that on any hymenium there are 50 per cent. of basidia of the  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$  type, 25 per cent. of the  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$  type, and 25 per cent. of the  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$  type.

Thirdly, with regard to the theoretical and actual positions of the spores of diverse sex, our Scheme III is very fairly satisfactory. (1) According to it, in the two-sex type  $(Ab)$ ,  $(Ab)$ ,  $(aB)$ ,  $(aB)$  there is only one possible arrangement, namely, where  $(aB)$  is diagonal to  $(Ab)$ . Observation showed that there were four basidia of this type and none with an arrangement such as we find in Scheme II, where  $(Ab)$  is diagonal to  $(Ab)$ . (2) Again, according to Scheme III, in the four-sex type  $(AB)$ ,  $(ab)$ ,  $(Ab)$ ,  $(aB)$  there are three possible ways in which the spores may be arranged, and not two only as shown in Scheme II, and the arrangement in which  $(ab)$  is diagonal to  $(AB)$  should occur twice as often as the arrangement in which  $(Ab)$  is diagonal to  $(AB)$ , and also twice as often as the arrangement in which  $(aB)$  is diagonal to  $(AB)$ . Of twenty basidia there were eight with  $(ab)$  diagonal to  $(AB)$ , four with  $(Ab)$  diagonal to  $(AB)$ , and eight with  $(aB)$  diagonal to  $(AB)$ . The theoretical numbers instead of being 8:4:8 should have been 10:5:5. The agreement, in view of the small numbers involved, is sufficiently satis-

factory. (3) Finally, according to Scheme III, in the two-sex type ( $AB$ ), ( $AB$ ), ( $ab$ ), ( $ab$ ) there is only one possible arrangement of the spores, namely, where ( $ab$ ) is diagonal to ( $AB$ ), and there should be no basidia where ( $AB$ ) is diagonal to ( $AB$ ). Observations showed that there were six with the expected arrangement with ( $ab$ ) diagonal to ( $AB$ ), and one with the unexpected arrangement with ( $AB$ ) diagonal to ( $AB$ ). For this last basidium Scheme III has no place.

We thus perceive that Scheme III accounts sufficiently well for all the experimental data obtained with thirty-one basidia, except for the one basidium last mentioned.

It is just possible, but not likely, that the spore tetrad of the exceptional basidium was not a true tetrad, but that two of the spores belonged to one basidium and two to another, the other four spores not having adhered to the cover-glass. It is also possible that in this particular basidium segregation of both pairs of sex factors, instead of taking place in the first division of the fusion nucleus as demanded by Scheme III, took place in the second division in the manner shown on the left-hand side of Scheme II.

Of the four possible modes of segregation which have been set forth above there can be no doubt that No. 4, illustrated by Scheme III, fits the observed facts far better than any of the others.

(5) *In some basidia segregation of both pairs of factors in the second division of the fusion nucleus, and in other basidia segregation of one pair of factors in the first division and of the other pair in the second division.* Assuming this type of segregation to take place, it might be represented by a scheme which would include the two outer columns of Scheme II and the two central columns of Scheme III.

The experimental facts fit the outer columns of Scheme III much better than the outer columns of Scheme II for reasons already set forth. Therefore our new scheme, as a whole, is not as good an explanation for the data obtained with the thirty-one basidia as Scheme III.

(6) *In some basidia segregation of both pairs of factors in the first division of the fusion nucleus, and in other basidia segregation of both pairs of factors in the second division.* Assuming this type of segregation to take place, it might be represented by a scheme which would be a combination of Schemes I and II.

The objections to Scheme II have already been discussed. In our new combination scheme the basidia of the four-sex type would be like those shown in the central column of Scheme II. This column has no place for four-sex basidia in which ( $AB$ ) and ( $ab$ ) are in diagonal positions, yet eight of the thirty-one basidia investigated were of this type. On this ground, in particular, our new scheme fits the experimental facts much less successfully than Scheme III.

(7) *In some basidia segregation of both pairs of factors in the first,*

*division of the fusion nucleus, in other basidia segregation of both pairs of factors in the second division, and in yet other basidia segregation of one pair of factors in the first division and of the other pair of factors in the second division.* Assuming this type of segregation to take place, it might be represented by a scheme which would be a combination of Scheme I, Scheme II, and the central part of Scheme III, or, since Scheme III includes Scheme I, it might be represented by a combination of Scheme II and Scheme III.

Scheme III, as already pointed out, except for one basidium, fits the experimental data very closely. The objections to Scheme II have already been discussed. A combination of Schemes II and III does not fit the experimental data as well as Scheme III by itself.

The experimental data can only be interpreted successfully on the supposition that the segregation of both pairs of factors does not take place in all the basidia in the first division of the fusion nucleus. If we accept Scheme III as the scheme most in accordance with the experimental data, we admit that segregation of one of the two pairs of factors frequently takes place in the second division of the fusion nucleus. Now, if one pair of factors frequently segregates in the second division, there seems no good reason why we should not admit that *both* pairs of factors may segregate in the second division. As may be seen by comparing the central columns of Schemes II and III, basidia of the four-sex type may arise in either of two ways: (1) by segregation of both pairs of factors in the second division of the fusion nucleus, and (2) by segregation of one pair of factors in the first division and of the other pair of factors in the second division. It will be remembered that of the forty-seven basidia investigated for basidial types twenty-five were four-sex and twenty-two two-sex. It is possible that not all of the twenty-five four-sex basidia became four-sex by the step-wise segregation shown in the centre columns of Scheme III, but that at least some of them became four-sex by segregation of both pairs of factors in the second division as shown in Scheme II. It is therefore possible that, although of all the schemes suggested Scheme III seems to fit the experimental data best, further investigation with a greater number of basidia might result in favour of a scheme which would represent segregation of the type No. 7. This scheme has this advantage over all the other schemes: it allows for all possible types of segregation, i.e. for segregation of both pairs of factors either at the first or at the second division, and for segregation of one pair of factors at the first division and of the other pair at the second division.

The occurrence of segregation of both pairs of sex factors in the second division of the fusion nucleus would explain the existence of the basidium of the  $(AB)$ ,  $(AB)$ ,  $(ab)$ ,  $(ab)$  type in which the two  $(AB)$  spores were found to have diagonal positions (cf. Scheme II). Segregation of both pairs of

sex factors in the second division of the fusion nucleus is not provided for in Scheme III, but it is to be expected in a combination of Schemes II and III such as that here under discussion. Thus the existence of the exceptional basidium may be taken as evidence which definitely favours the combination scheme. However, if segregation of both pairs of factors at the second division of the fusion nucleus takes place at all, the evidence so far obtained seems to show that such a mode of segregation is comparatively rare.

Finally, it may be remarked that, whatever the exact steps in the segregation of the two pairs of sex factors in *Coprinus lagopus* may be, it seems impossible to explain the experimental data unless one accepts the view that the disjunction of homologous chromosomes may take place either at the first or at the second of the two divisions of the fusion nucleus.

### *Coprinus Rostrupianus*.

*Coprinus Rostrupianus* is a species in which the spores on any individual fruit-body fall into two opposite sexual groups. We may suppose that the bisexuality depends on the presence in the fusion nucleus of a single Mendelian pair of factors ( $Aa$ ), and that the nucleus of each spore bears either the ( $A$ ) or the ( $a$ ) factor, but not both. Every basidium bears two ( $A$ ) spores and two ( $a$ ) spores (10).

If segregation of the sex factors takes place always in the first division of the fusion nucleus of *C. Rostrupianus*, then the two ( $A$ ) spores should have adjacent positions in every spore-tetrad. If, on the other hand, segregation takes place always in the second division, the two ( $A$ ) spores should occupy adjacent positions in some basidia and diagonal positions in others.

Up to the present, as regards sex, the positions of the spores have been determined for six basidia of *C. Rostrupianus*. The two ( $A$ ) spores had adjacent positions in four of the six basidia and diagonal positions in the other two. This evidence points to the segregation process taking place in *C. Rostrupianus* at least sometimes during the second division of the fusion nucleus. Further work on this species is in progress.

### VII. SUMMARY.

1. As found by Hanna (5), on the hymenium of any individual fruit-body of *Coprinus lagopus* there are four sexually different types of spores, each of which bears two sex factors, one belonging to one Mendelian pair of factors and the other belonging to another Mendelian pair of factors, so that the spores may be represented by the symbols ( $AB$ ), ( $ab$ ), ( $Ab$ ), and ( $aB$ ).

2. Three types of basidia, and three only, are present in the hymenium

of any fruit-body of *Coprinus lagopus*, and they may be represented by the sex factors of their spores as follows: a four-sex type ( $AB$ ), ( $ab$ ), ( $Ab$ ), ( $aB$ ); a two-sex type ( $AB$ ), ( $AB$ ), ( $ab$ ), ( $ab$ ); and another two-sex type ( $Ab$ ), ( $Ab$ ), ( $aB$ ), ( $aB$ ).

3. Of the three types of basidia in the hymenium of any fruit-body of *Coprinus lagopus* 50 per cent. are of the type ( $AB$ ), ( $ab$ ), ( $Ab$ ), ( $aB$ ), 25 per cent. of the type ( $AB$ ), ( $AB$ ), ( $ab$ ), ( $ab$ ), and 25 per cent. of the type ( $Ab$ ), ( $Ab$ ), ( $aB$ ), ( $aB$ ).

4. By means of the cover-glass-contact and dry-needle methods the spores were removed from thirty-one basidia and sown singly in culture media, a record being kept of the actual positions the spores had had on their respective basidia. Then, by mating the monosporous mycelia in Petri dishes and employing the clamp-connexion criterion, the sexes of the spores were determined. The actual positions of the spores of diverse sex on each one of the thirty-one basidia thus became known. These basidia were all derived from fruit-bodies which appeared on a mycelium developed from spores liberated by a single wild fruit-body.

5. For any individual fruit-body of *Coprinus lagopus* the existence of four-sex basidia as well as two types of two-sex basidia, the numerical ratio in which the three types of basidia occur, and the actual positions of the spores of diverse sex on the individual basidia, are best explained on two assumptions: (1) that the two sex factors in the nucleus of each spore are carried by two separate chromosomes, one factor being carried by one chromosome and the other factor by the other chromosome; and (2) that in some basidia the segregation of both pairs of sex factors takes place in the first division of the fusion nucleus of the basidium, while in other basidia segregation of one pair takes place in the first division and of the other pair in the second division. If in some basidia segregation of both pairs of sex factors takes place in the second division of the fusion nucleus, this mode of segregation is relatively infrequent.

6. In *Coprinus lagopus* it is inferred from the experimental data that the disjunction of homologous chromosomes may take place either at the first or at the second of the two divisions of the fusion nucleus.

7. In *Coprinus Rostrupianus*, in which the spores on any fruit-body fall into two opposite sexual groups, and where each basidium produces two spores with an ( $A$ ) sex factor and two with an ( $a$ ) sex factor, the positions of the spores as determined for six basidia indicate that in this species segregation of the sex factors takes place at least sometimes during the second division of the fusion nucleus.

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